

# **RESPONSIVITY: STUDIES ON STRESS-RELATED PROCESSES**

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## **Abstract**

The theory of allostatic load suggests that in certain individuals cumulative strain could lead to physiological dysregulation and impaired health. This thesis presents a series of four studies investigating novel aspects of cardiovascular and neuroendocrine stress-mechanisms across the adult age spectrum. Studies 1 and 2 involved a community sample of 139 adults aged 65–80 years. In Study 1, the relationship between cortisol, heart rate and blood pressure responsivity and performance of cognitive tasks was assessed. It was found that low cortisol responsivity and better cardiovascular recovery were related to superior memory performance but not to variations in reasoning task performance, suggesting a specific association with memory. Study 2 investigated psychosocial factors associated with cortisol regulation in everyday life. It was found that gender and low socioeconomic status were independently related to cortisol responses to waking. Study 3 explored whether biological stress responsivity precedes ill-health in those most at risk. 103 young adults were exposed to laboratory stress tasks, and data were collected on cardiovascular, cortisol and interleukin (IL) 6 responses. Participants' parents provided information on family history, and a two-generation, family history risk score was computed. Results indicate that participants with a positive family history of cardiovascular disease had greater blood pressure responses to the tasks, and that this relationship was independent of their own cardiovascular, smoking and weight status. An association between family risk and IL-6 responses was also observed. The results suggest that aberrant patterns of stress reactivity are present in young people at risk before the onset of any health problems in themselves. The final study explored the bidirectional nature of the relationship between physiological responsivity and psychological functioning by investigating the effects of an inflammatory stimulus (typhoid vaccination) in a double-blind, placebo-control design. The results indicated that increases in IL-6 were correlated with negative mood induction. The findings of these studies have implications for understanding the contribution of stress-related processes to health.

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## List of Abbreviations

5H1AA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
5HTTLPR	5-hydroxytryptamine transporter gene linked polymorphic region
11 $\beta$ -HSD	11-beta hydroxysteroid dehydrogenase
ACTH	adrenocorticotrophic hormone
ADR	adrenergic receptor
Ag	silver
Ag/Cl	silver chloride
Ala	alanine
AMS	ambulatory monitoring system
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ANS	autonomic nervous system
Arg	arginine
BMI	body mass index
bpm	beats per minute
CAR	cortisol awakening response
CES-D	Centre for Epidemiological Studies Depression Scale
cm	centimetre
CNS	central nervous system
CO	cardiac output
CRH	corticotrophin releasing hormone
Cys	cysteine
CVD	cardiovascular disease
DBP	diastolic blood pressure
DHEA	dehydroepiandrosterone
DNA	deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
Dz	impedance change
Dz/Dt	impedance change first derivative
ECG	electronic cardiogram
EDTA	ethylenediaminetetracetic acid
ELISA	enzyme linked immunosorbent assay
FH	family history
GABA	gamma-aminobutyric acid
Gly	glycine
GR	glucocorticoid receptor
h	hour
HACER	hypothalamic area controlling emotional responses
HPA	hypothalamic-pituitary-adrenocortical
HR	heart rate
HRV	heart rate variability
Hz	Hertz

IL	interleukin
IL-1Ra	interleukin-1 receptor antagonists
ICG	impedance cardiogram
INF	interferon
IBI	inter-beat interval
k $\Omega$	kilo-Ohm
kg	kilogram
kHz	kilo-Hertz
LOT	Life Orientation Test
LSD	least significant difference
LVET	left ventricular ejection time
M $\Omega$	mega-Ohm
mA	milli Ampère
MAP	mean arterial pressure
mg	milligram
min	minute
mmHg	millimetre of mercury
ms	millisecond
MR	Matrix Reasoning
ng/ml	nanogram per millilitre
nmol/l	nanomoles per litre
PEP	pre-ejection period
pg/ml	pictogram per millilitre
POMS	Profile of Mood States
rpm	revolutions per minute
RPP	rate pressure product
SAM	sympathetic-adrenomedullary system
sd	standard deviation
SBP	systolic blood pressure
SES	socioeconomic status
SV	stroke volume
TAQ	Task Appraisal Questionnaire
Thr	threonine
TNF $\alpha$	tumour necrosis factor
TPR	total peripheral resistance
UK	United Kingdom
USQ	Undergraduate Stress Questionnaire
VPA	Verbal Paired Associates
VU	Vrije Universiteit
WAIS	Wechsler Adult Intelligence Scale
WHR	waist to hip ratio
WMS	Wechsler Memory Scale
xg	acceleration due to gravity
Z0	thoracic impedance

# **Chapter 1: Literature Review**

## **1.1 Introduction**

Stress is a broad, high-level construct that has become ubiquitous in modern society. Established by Selye in 1936, the term was first defined as the non-specific response of the body to any demand made upon it. This was based on the notion that individuals possess a certain tolerance to stress, but when placed under excessive strain illness can ensue. With the recognition of stress as a biopsychosocial construct, it is no longer appropriate for this term to be defined purely in terms of physiological stimulus and response. As a result, most contemporary frameworks of research adopt the transactional model (Lazarus & Folkman, 1984). This comprehensive approach takes account of the dynamic person-environment relationship and considers that a stress response arises through interactions between individual demands and psychosocial resources; thus, psychophysiological responses are elicited when the persons' adaptive capacity fails to match demands, and as a result illness may occur. This chapter will consider each of these approaches whilst also suggesting an alternative approach, the theory of allostatic load, which highlights the physiological aspects of the stress response.

The view that stress can cause illness is not new, however, some still argue that the relationship remains unproven. Herein, this report will describe a series of four studies, each investigating a particular aspect of the stress-health construct. The thesis will adopt a diverse methodology evaluating different populations and health endpoints across the life course. The main focus of this work will be physiological mechanisms, which mediate the relationship between chronic strain and illness.

## **1.2 Components of the Stress Response**

When one encounters a stressful situation a series of responses are activated. These responses involve changes in four general domains that are complexly integrated but do not necessarily change in parallel. For practical purposes these are discussed in terms of cognitive, emotional, behavioural and physiological effects.

### **1.2.1 Cognitive Response to Stress**

Cognitive responses to stress include changes in perception, attention, memory and decision-making. Attentional processes are particularly vulnerable and failure to notice important but peripheral stimuli under stressful conditions is a common factor in poor human performance. Traditionally the relationship between stress and cognition has been construed as an inverted-U with suboptimal performance at very low or very high levels of stress. However, it is now thought that this is an over-simplification, since different types of patterning have different effects on speed of processing, attention and short-term memory. For example, memories of stressful events are often incomplete, while others have an ability to maintain performance under stressful situations but cognitive ability suffers once the stressors has terminated. Research now focuses on the vast individual variation that accompanies the cognitive stress response.

### **1.2.2 Emotional Response to Stress**

The emotional response to stress involves feelings, which include distress, anxiety, fear and depression. These are the core emotions that people tend to associate with a psychosocial stressor; however, there is generally no accepted measure of the subjective emotional stress response. This is partly because stress overlaps with other

moods, so separate measures of stress would be somewhat redundant. This omission does present methodological difficulties, since people vary greatly in their emotional responses. Consequently, any particular scale may not capture the subjective experience of all the people exposed to the same stressor.

It has frequently been found that although stressors elicit subjective as well as physiological responses, the two do not correlate well. For instance, a meta-analysis of nine experiments evaluating the association between cardiovascular and emotional responses found that the relationship between these factors was positive but small (Feldman et al, 1999). This suggests that physiological and emotional responses occur together but that the effects of reporting bias need to be considered.

### **1.2.3 Behavioural Response to Stress**

Behaviour changes are central to the stress response. Traditionally these behaviours were documented in terms of common animal actions such as the fight or flight response, with each set of behaviours underpinned by a physiological adjustment supporting the energy demands of the action. Now health psychology tends to focus on behaviours that are associated with well being. These can include alterations in smoking, alcohol and food consumption, sleep disturbances and physical activity. At one extreme, stress contributes directly to major health problems such as alcohol dependence and eating disorders, while at the other extreme, the influence of stress, particularly when chronic, can be difficult to disentangle from many other social, cultural and psychological factors that are also related to these actions. In addition, there is great individual variability in the behavioural stress response with, for example, some people becoming increasingly inactive when stressed, while others may tend to increase exercise levels during stressful periods. The behavioural response is also strongly associated with all of the other aspects of the stress response. That is, stress



can alter one's decision to partake in healthy behaviours and subsequent health damaging behaviours may then go on to alter physiological responses to stress.

### **1.2.4 Physiological Response to Stress**

When an individual perceives a stressful event, a series of physiological changes occur. Although it is recognised that all aspects of the stress response are integrated, this thesis is especially concerned with the individual psychobiological alterations that can occur when one is stressed. As a result a more detailed description of this process is provided. Activation of the stress system initially leads to beneficial behavioural and physical alterations. However, if protecting forces fail to adequately control elements of the stress response the process becomes harmful and pathology may develop. The biological pathways mediating the effects of stress can be subdivided into three main areas; neural transmission, neuroendocrine transmission and immune system impairment. These three areas will be focused on in more detail throughout the thesis.

#### ***1.2.4.1 Stress and Autonomic Function***

The sympathetic and parasympathetic branches of the autonomic nervous system (ANS) have for decades been considered key putative pathways linking stress to pathological processes. With these two branches of the ANS working reciprocally, the sympathetic branch controls activation and energy expenditure while the parasympathetic division directs energy restoration and activity reduction. When an individual is exposed to a stressor the sympathetic-adrenomedullary system (SAM) is activated. This system is responsible for the release of adrenaline and noradrenaline, which mobilise the body's resources by increasing the rate of glycogenolysis and cardiovascular activity. Catecholamines are difficult to measure since responses are rapid and transient, and concentrations in venous blood may not be representative of the

overall level of circulating adrenaline and noradrenaline (Grassi et al, 1999). As a result, changes in cardiovascular function are most often investigated as reliable biological markers of the stress response. These stress-related cardiovascular processes include increased heart rate, stroke volume, blood pressure and decreased pre-ejection period (time interval between onset of ventricular depolarisation and the opening of the semi-lunar valves) to ensure that oxygen is transported efficiently to essential target organs (Sherwood et al, 1990; Cacioppo et al, 1995; Clow, 2001; Vrijkotte et al, 2004). Problems for health arise when the stress response is repeatedly activated so that the cardiovascular system suffers from unnecessary deterioration. For example, it has been observed that high heart rate may be an independent risk factor for coronary artery disease (Dyer et al, 1980; Uden et al, 1991), while minimum heart rate achieved over a 14-hour monitoring period is positively associated with coronary atherosclerosis (Perski et al, 1992). Similarly, stress-induced blood pressure elevations, over time can lead to endothelium damage, atherosclerosis and possibly myocardial infarction (Muller & Tofler, 1990; Manuck et al, 1995) or stroke (Chambless et al, 2004). Prolonged pre-ejection period has also been found in patients with aortic stenosis and primary pulmonary hypertension (Shigematsu et al, 1988), although studies relating stress, pre-ejection period function and disease are relatively rare. The relationship between stress, pre-ejection period and health is investigated in Studies 1 and 3. In addition, stress-related increases in heart rate and blood pressure have been related to learning (Cohen et al, 1973; Cohen et al, 1980) and memory impairment (Waldstein, 2003), although, again studies investigating stress, autonomic function and cognition are less common. This issue is explored in Study 1.

The autonomic stress response is also characterised by alterations in parasympathetic activity. High levels of parasympathetic activity or vagal tone act in

opposition to sympathetic traffic, and have traditionally been studied in terms of heart rate variability. Heart rate variability is the measure of the cyclic variation in heart rate, which is influenced by blood pressure and respiratory processes. These fluctuations in intervals between normal heartbeats provide information about autonomic regulation and because the heart is innervated by both the sympathetic and vagal fibres they give rise to different frequencies. Identification of these frequencies can be used as markers of cardiac autonomic control (Stein & Kleiger, 1999). Three distinct rhythms have been identified within beat-to-beat modulation of the heart; a high frequency component (0.15-0.4 Hz), a low frequency component (0.04-0.15 Hz) and a very low frequency component (0.0033-0.04 Hz) (Berntson et al, 1997). Studies of heart rate variability in response to stress have primarily focused on the high frequency component. This component, often referred to as respiratory sinus arrhythmia, is associated with respiratory signal and mediated by vagal tone only. The inability to adapt well to stress is therefore associated with deviation from the normal oscillatory mode, decreased complexity of cardiac signal and reduction in observed heart rate variability. Changes in heart rate variability have been characterised in many cardiovascular disorders, including hypertension (Singh et al, 1998), sudden cardiac death (Goldberg et al, 1990), coronary heart disease (Peng et al, 1995; Dekker et al, 2000), ventricular arrhythmia (Rosenbaum et al, 1994) and myocardial infarction (Bigger et al, 1992). Heart rate variability has also been used to predict mortality and morbidity after myocardial infarction (Wijbenga et al, 1998), congestive heart failure (Saul et al, 1991) and risk of rejection after cardiac transplantation (Binder et al, 1992). Similar alterations in heart rate variability are seen in many non-cardiac pathologies such as diabetic neuropathy (Lischner et al, 1987), asthma (Lehrer et al, 1996), sudden infant death syndrome (Pincus et al, 1993) chronic fatigue syndrome (Pagani et al, 1994), panic and general

anxiety disorder (Lyonfield et al, 1995; Yeragani et al, 2004), major depression (Rechlin et al, 1994) and anorexia nervosa (Kreipe et al, 1994). Despite a wealth of evidence linking lowered heart rate variability to morbidity and mortality, few studies have examined the association between stress, heart rate variability and health (Ruediger et al, 2004). This relationship is investigated in Studies 1 and 3.

#### ***1.2.4.2 Stress and Neuroendocrine Function***

Another widely studied pathway through which stress can exert its effect upon health involves neuroendocrine transmission via the hypothalamic-pituitary-adrenocortical axis (HPA axis). It is known that cerebral cortical interpretation of threat to homeostasis (such as that experienced during stress) can result in activation of the paraventricular nucleus of the hypothalamus. In response, corticotrophin releasing hormone (CRH) is secreted from the median eminence of the hypothalamus. CRH, by transportation through the hypothalamic hypophyseal portal system, then initiates the synthesis of adrenocorticotrophic hormone (ACTH) and beta endorphin in the anterior pituitary. ACTH in turn stimulates the production of steroid hormones in the adrenal cortex via the circulatory system. Although many other hormones are also influenced by the HPA axis (e.g. androgens, oestrogens, mineralocorticoids) it is the glucocorticoid response, which has received the most attention in stress physiology research. The glucocorticoid cortisol has wide spread effects on the periphery (these include increases in metabolism, catecholamine synthesis, gluconeogenesis, water diuresis, sodium retention, lipolysis) which can ultimately go on to impair functioning.

Under basal conditions, cortisol secretion exhibits a 24 hour diurnal profile in which concentrations present a morning maximum followed by a continuous decline over the course of the day until a trough is reached around midnight. Within the first 30 minutes of awakening, free cortisol levels rise by approximately 50-150 % and remain

elevated for at least 60 minutes (Pruessner et al, 1997). This is commonly known as the cortisol awakening response. Importantly the response is independent of age, quality and length of sleep, postural shift, use of an alarm clock and physical activity (Clow et al, 2004). The cortisol awakening response is also reported to be consistent over similar situations but altered by illness and stress (Schulz et al, 1998; Pruessner et al, 1999; Stone et al, 2001). However, to date, few studies have examined whether the cortisol awakening response mediates the influence of stress on health.

In addition to the spontaneous release of cortisol over the day, cortisol is also secreted in response to stress, and stress-induced activation of the HPA axis is superimposed upon this background activity. Typically cortisol levels rise sharply 20 minutes after an acute stressor and will usually return to normal within one hour of an acute rise. Cortisol production is regulated through negative feedback to the hippocampus, hypothalamus and pituitary gland and acts via intracellular receptors in these regions. The binding of cortisol to type I and type II receptors, over time, induces a conformational change in the receptor, which results in a dissociation of the receptor from its attached protein. Such reactions have important consequences for physical, cognitive and psychological functioning. For instance, prolonged cortisol exposure can lead to muscle atrophy, decreased sensitivity to insulin, risk of diabetes, increased fat deposition, hypertension, hyperlipidemia, hypercholesterolemia, coronary artery disease, amenorrhea, immunosuppression (Meaney et al, 1991). In addition, prolonged increases in cortisol levels have been related to psychological and cognitive dysfunction, including major depression (Blackburn et al, 1987), anxiety disorders (Hubert & De Jong-Meyer, 1992), eating disorders (Piran et al, 1985) and memory impairment (Sauro et al, 2003; Lupien et al, 2005).

#### **1.2.4.3 Stress and Immune Function**

The immune system is the body's primary defence against infection and invading pathogens and consists of two main branches; humoral and cellular immunity. Humoral immunity is involved in the defence against bacteria and viruses in bodily fluids, while cell-mediated immunity is relevant to intracellular viruses, cancer cells and transplantation tissue. Serum antibodies or immunoglobulins mediate humoral immunity. These are proteins that derive from B-lymphocytes in the bone marrow and react with specific antigens. Cellular immunity involves T-lymphocytes that arise in the bone marrow and mature in the thymus before circulating in the blood and lymph. T-cells do not recognise antigens by themselves, so these are usually presented by macrophages. Once activated T-cells acquire 'memory', migrating to tissues in which they are most likely to re-encounter their specific activating antigen. T-cells are differentiated into various sub-types and the actions of these are mediated through cytokines. Cytokines are glycosylated and non-glycosylated polypeptides, which act as intercellular signalling proteins in the immune system. Secreted by white blood cells and virtually all nucleated cells, cytokines have immune modulating effects that are understood to control most of the physical and psychological symptoms associated with infection and inflammation; as such they have become increasingly important in understanding the relationship between stress and health. There are three broad classes of cytokines; class 1 (lymphokines and monokines), class 2 (growth factors and colony-stimulating factors) and class 3 (chemokines). Class 1 cytokines have received most attention in the psychobiology literature and are considered herein.

Lymphokines and monokines are released from lymphocytes and monocytes, respectively. Included in these are the sub-categories of interleukins, tumour necrosis factor and interferons. Interleukins have the ability to communicate between various

white blood cell populations and comprise multiple antagonist and agonist isoform subtypes. For example, interleukin-1 (IL-1 $\alpha$ , IL-1 $\beta$ ) is responsible for the attraction of macrophages and neutrophils to the site of inflammation, it is also responsible for stimulating B cells to produce antibodies, stimulating natural killer cells to destroy foreign cells, stimulating endothelial cells to release peptides that increase vessel permeability and diameter, and stimulating T-cells to produce more cytokines. In addition, tumour necrosis factor (TNF $\alpha$ , TNF $\beta$ ) is responsible for destroying tumour cells, stimulating phagocytosis, releasing IL-1, IL-2 and IL-6 and causing cachexia during chronic inflammation. Lastly, interferons (INF- $\alpha$ , INF- $\beta$ , INF- $\gamma$ ) inhibit viral replication and increase the expression of major compatibility antigens (Maier & Watkins, 1998; Corwin, 2000a).

Due to their potency and fundamentality in the immune response, cytokines now have a particular importance in the work on stress and immune function. During the response to various stressors encountered by the individual, including normal growth demands, infection by micro-organisms, injury, inflammation and psychological distress, cytokine production is seen to increase dramatically (Dinarello, 1999; Gabay & Kushner, 1999). This dysregulation of the cytokine cellular system has significant implications in the development of a variety of disorders (Corwin, 2000b) including heart failure (Dibbs et al, 1999; Kapadia, 1999), osteoporosis (Poli et al, 1994; Angeli et al, 1999; Roodman, 1999), asthma (Wong et al, 2001), rheumatoid arthritis, type I diabetes mellitus, multiple sclerosis, inflammatory bowel disease, lupus (Ward, 1995; Workman, 2000), depression and sickness behaviour associated with fever (Larson & Dunn, 2001; Kelley et al, 2003). Beside the increase of cytokine production during periods of stress, future work needs to consider the causal nature of this relationship;

this issue is address is study 4. A related aspect of this is the interaction between autonomic, neuroendocrine and immune systems.

#### ***1.2.4.4 Stress, Autonomic, Immune and Neuroendocrine System Interaction***

When investigating the psychophysiological stress response, one must consider that the neuroendocrine and immune systems do not act alone and are, in fact, mutually interactive (Black, 1994a; Black, 1994b; Straub et al, 2000). The evidence that immune and neuroendocrine mechanisms can affect each other has been classified by Besedovsky and Del Rey (1996) as follows: first, immune, endocrine and neural cells can express receptors for cytokines, neurotransmitters and hormones. For example, cortisol receptors exist in the cytoplasm of lymphocytes and glucocorticoids have been found to inhibit expression of cytokine receptors. Second, there is evidence that immune and neuroendocrine products exist in lymphoid, endocrine and neural tissue. Third, endocrine and neural mediators have been shown to affect the immune system. For example, glucocorticoids suppress the activation of circulating lymphocytes and cortisol inhibits the production of cytokines (Chrousos, 2005). The sympathetic nervous system also innervates immune organs such as the spleen and lymph nodes, while corticosteroids have long been used as anti-inflammatory agents. Fourth, there is evidence that the immune system exerts an effect on stress-responsive neuroendocrine systems. Less is known about this process but it has been shown that  $\text{TNF}\alpha$ , IL-1 and IL-6 can stimulate the HPA axis alone or in synergy possibly by activation of central catecholamine pathways (Tsigos & Chrousos, 1996). The evidence and importance of these interactions means that research must endeavour to consider all aspects of the stress response in relation to health.



#### ***1.2.4.5 The Bi-directional relationship of Stress, the Central Nervous System and Immune/Neuroendocrine Systems***

Until this point, evidence presented in this chapter has implied a basic linear relationship between stress appraisal, physiological responsivity and pathological outcome. However, this view is overly simplistic. The existence of a bi-directional association between stress and health therefore needs to be considered. To understand this interaction, one needs to be aware that the central nervous system comprises two separate components that are involved in the stress response; the first being structural elements such as the hypothalamus, limbic system and cerebral cortex, the second being regulatory systems such as the HPA axis, autonomic nervous system and SAM. This bi-directional relationship between the central nervous system and regulatory systems implies that stress appraisal centred in higher order brain areas such as the limbic system affects the immune system through the HPA axis. In contrast, the immune system, by cytokine elevation, may also affect these same brain regions through the HPA axis (Besedovsky & Del Rey, 1996).

There is growing recognition that the immune system does not act in isolation and that communication pathways exist between the inflammatory response and the brain, often resulting in *sickness behaviour* (Kop & Gottdiener, 2005). This includes feelings of malaise, fever, anorexia, anhedonia, impaired cognition and concentration, and depressed mood (Dantzer, 2001; Konsman et al, 2002; Kelley et al, 2003; Dantzer, 2004). However, the mechanisms by which cytokine activation affects the brain are not fully established. Watkins et al (1995) proposed four possible pathways. First, entry of cytokines into the brain may occur at circumventricular sites lacking a blood-brain barrier. The blood-brain barrier effectively prevents passage of substances into the brain via non-specific mechanisms. However, there are a few regions in the brain,

known as circumventricular organs where fenestrated capillaries are found, allowing plasma passage to occur. Cytokines could passively cross into the brain at these areas (Stitt, 1990; Katsuura et al, 1990). Second, cytokines may bind to and affect the metabolism of cerebral vascular endothelium, thereby inducing the generation of central mediators. Such metabolic effects can alter the permeability of the blood brain barrier to other substances. For instance, cytokines may act on brain endothelial cells which activate cyclooxygenase, enabling the synthesis of prostaglandins, potentially allowing passage into the brain of illness mediators that would otherwise be excluded by the blood brain barrier (Cao et al, 1996; Cao et al, 1997). Third, it has been proposed that cytokines gain access to the blood brain barrier via carrier mediated transport (Banks & Kastin, 1991). Evidence confirms that cytokines reach the brain in greater quantities than would be predicted by passive diffusion; while transport of radiolabelled IL-1 is blocked by administration of IL-1 receptor antagonists (Banks et al, 1994).

Each of the three pathways mentioned requires cytokines to reach the brain via humoral means. However, sickness behaviour can occur in the absence of detectable blood cytokine levels (Kluger, 1991). In light of this evidence a fourth, neural route of communication is proposed. The vagus nerve represents the main sensory pathway from the abdominal organs to the brain, while afferent neurones innervate the body site where infection takes place. In this case local increase in tissue levels of cytokines would be sufficient to signal the brain. Specifically, evidence suggests that activation of subdiaphragmatic vagal afferents mediates a wide range of illness responses produced by IL-1 $\beta$  and TNF $\alpha$ . The vagus is also known to contain a very large number of sensory afferent fibres (Ritter et al, 1992). The vagus nerve projects to the nucleus of the solitary tract and the parabrachial nucleus of the brain stem and from there to the paraventricular nucleus of the hypothalamus, the thalamus, central and basolateral

amygdala and cerebral cortex. The amygdala is primarily responsible for motivation and emotional behaviour, such as depressed mood. Cytokine-induced activation of the vagus or entry to the brain across the blood brain barrier may therefore have profound effects on behaviour. At present much of the evidence examining the bi-directionality of physiological responsivity and health is associated with clinical samples and illness-induced cytokine elevations. The key issue now is to explore whether cytokines communicate with the brain in the absence of illness. This problem is investigated further in Study 4, Chapter 5.

### **1.2.5 Section Summary**

This section has outlined the four broad areas, which typically define the stress response. Although these are often divided into simplified categories and considered separately it must be emphasized that the responses occur in a highly complex and integrated way. By considering all four types of response in unison stress research is attempting to move away from the traditional linear biomedical model of stress towards a more integrated biopsychosocial approach. This perspective attempts to combine the psychological (i.e. cognitive and behavioural), with social (e.g. environmental demands) and biological / physiological aspects of the stress response. By considering all aspects of the stress response in unison a broader picture of stress and health is available whereby all four stress responses affect and are affected by a person's individual health status. In this way the individual can be considered as a complete but nevertheless complex system. From this, several theoretical models have been constructed from which stress research can be based. These are considered in the following section.

## **1.3 Models of Stress**

Theoretical models of stress have been based, mostly, on the four types of response outlined above. The extent to which these responses are included in each of the models and the emphasis each place on their interaction will be considered herein.

### **1.3.1 Stimulus Based Models**

The main stimulus-based model of stress is the life change approach. This model defined stress as the amount of adjustment or life change with which a person was faced. This approach suggested that any life change would tax resources and was, therefore, likely to be detrimental to health and well being. Originally it was characterized by using checklists of life event and the number and type of life event a person experienced over a given period of time determined an individual's level of stress. The most widely used measure of life events was the Social Readjustment Rating Scale (Holmes and Rahe, 1967), which listed 43 events that were weighted for average stress on a scale of 1 to 100.

The major advantage of the life change approach has been in its definition of stressful experiences as quasi-objective phenomena. This has made it theoretically possible to distinguish exposure to adverse experiences from the emotional responses they engender, making it possible to discover whether stress exposure precedes illness even in retrospective studies. However, as a measurement tool, the checklist method has been widely criticized (Turner & Wheaton, 1995). First there is a problem of comprehensiveness and individual interpretation. For example, it is impossible to create a checklist where every possible stressor for every individual is included; as a result certain stressor for some people may be omitted. Items are also open to considerable individual variation in interpretation and statements like 'change in financial situation'

are ambiguous. There are also concerns about the weighting system whereby uniform scores are given to every one for a particular event, even if that event is not possible (for example, 'death of a spouse'). Second, there are problems with the retrospective nature of the model's assessment, which has obvious implications for understanding the causal link between stress and health. Third, checklist response are summed, which infers that life stress is cumulative, when it is possible that one life event could be offset by or interact with another. Similar to this, the model places a great deal of emphasis on social aspects of the stress response but does not consider many of the physiological or behavioural aspects of stress and health. The model is therefore criticised for its lack of consideration of all aspects of the stress response and as a result has fallen out of favour among many stress researchers.

### **1.3.2 Response-Based Models**

Response based models have concentrated on the physiological components of the stress response. Canon (1914) was the first to detail the response of the autonomic nervous system to stressful stimuli. This was labelled the 'fight or flight' response. The response prepares the body for confrontation or escape and argues that the psychological changes were structured around preparing the organism for vigorous physical activity. This accounts for increased breathing rate and depth, for example, with the onset of stress. Selye (1956) expanded Cannon's work to incorporate the HPA axis, and centered his work on corticosteroids. Selye proposed the General Adaptation Syndrome to describe three phases of the physiological stress response. In the first phase, 'alarm', the body activates the fight-flight response to deal with the stressor. In the second phase, 'resistance', the body attempts to restore homeostasis and reach maximum adaptation to the stressor, but may remain in a state of higher arousal than normal. In the continued presence of the stressor, however, the final stage,

‘exhaustion’, can occur when physiological resources are over stretched and breakdown resulting in disease and death can occur.

Cannon and Selye’s approaches are similar in that they both use a homeostatic model of the physiological model of the stress response where the body attempts to control equilibrium. In addition, they both defined stress as a non-specific physiological response. There are a number of problems with these assumptions. First, although the model considers physiological aspects of the stress response (unlike stimulus-based approaches), these approaches fail to say exactly how exhaustion and illness will occur, for example, there is no mention of what processes are actually involved in this process or whether the effects are instantaneous or occur over a longer period of time. Second, by concentrating on the physiological aspect of the stress response the model also fails to take into account other important aspects of the stress process which include ones cognitive, behavioural and emotional response to that stressor. In this way response-based approaches simply define the person as a biological system. This criticism also relates to the third point, in that physiological responses are not non-specific, and vary greatly between stressor and individual. For example, it has been found that not every individual will react the same way to every given situation; response is, therefore, partly determined by individual interpretation of events (their cognitive and emotional response) which will go onto determine physiological reaction.

The work stimulated by Selye and Cannon has been important in forwarding understanding of biological stress responses. However, this approach tends to neglect the emotional, behavioural and cognitive response, which completes the stress process.

### **1.3.3 Interaction-Based Models**

Interactional approaches to stress emphasise individual differences in perceived stress and the importance of psychological processes. Interactional approaches to stress

also propose that it is the interplay between emotional, cognitive, behavioural and physiological responses, which are critical in determining health outcome. Two main interactional theories will be considered. The first, the transactional model, is a more traditional approach which while acknowledging the importance of all aspects of the stress response tends to focus most heavily on the cognitive domain. The second is a relatively new model, the theory of allostatic load which counterbalances the more traditional interactional theories by examining the importance of the physiological stress response in a psychosocial context.

### ***1.3.3.1 Transactional Approach***

The transactional approach to stress postulates that various factors involved in the stress response can influence each other and act as both independent and dependent variables. The dominant transactional model was developed by Lazarus and Folkman (1984). This comprehensive approach takes account of the dynamic person-environment relationship and considers that a stress response arises from interactions between individual demands and psychosocial resources; thus, psychophysiological responses are elicited when the persons' adaptive capacity fails to match demands, and as a result illness may occur. Although the model considers all aspects of the stress response cognitive appraisal is central to the approach. The model proposes that when an event occurs, individuals go through three stages of appraisal. The first stage, *primary appraisal* is where the demands of the event on the individual are evaluated. The second stage is *secondary appraisal* where the person evaluates whether the resources available will enable them to cope with the demands. Available resources can be environmental (e.g. financial and social support) or personal (e.g. personality type and past experience). It should also be noted that these resources may also influence primary appraisals, thus primary and secondary appraisals do not necessarily occur in a

linear or sequential fashion, but influence each other and may occur in parallel. As a result of this process an event can be evaluated as *irrelevant*, (i.e. not relevant to the persons well being); *benign-positive* (i.e. positive and / or not threatening) or *stressful*. Stressful appraisals can then be broken down into those that involve harm, loss, challenge or threat, although these categories are not mutually exclusive.

This model has stimulated a substantial amount of research, much of which supports the role of appraisal in modulating subjective and physiological stress responses. However, the model has several limitations, many of which question the central role allocated to the cognitive appraisal process (Zajonc, 1984). One of the difficulties of measuring appraisals as part of a dynamic process means it is hard to distinguish appraisals from cognitive processes that are part of the stress response itself. In this way the model is almost untestable. It is unfalsifiable, given that almost any empirical results concerning the effect (or lack of effect) of stress on disease may be explained by proposing a particular combination of demands, resources and vulnerabilities. This approach is also based on the notion that a few single highly stressful events will lead to health impairments; this is problematic as the approach fails to take into account the cumulative effect of stressful events over time. In addition the model focuses on the fact the individuals are constantly appraising their environment in a conscious manner. However, there are undoubtedly situations where conscious appraisal does not take place, which the model fails to account for.

The approach also fails to elaborate on the possible physiological mechanisms, which are said to mediate the relationship between appraisals and stress. It, therefore, does not propose how situation appraisals actually lead to the numerous disorders, which have over recent years been associated with stress. Therefore, although the transactional approach to stress has greatly increased understanding of individual



differences in the stress response, and the role of cognitive factors in this process, it is not considered a complete framework, because it has not elaborated on the physiological mechanisms of the stress response. This has led to alternative models, which seek to redress this balance. The last model to be considered, consequently, approaches the stress process in an integrated way but places a greater emphasis on physiological aspects of the stress response. It is hoped that by exploring this alternative approach a more complete understanding of the stress process can be gained.

### ***1.3.3.2 Theory of Allostatic Load***

The theory of allostatic load centres on the notion that cumulative strain will eventually lead to physiological dysregulation and ultimately impaired health. The model centres predominantly on the physiological stress response, but at the same time considers that emotional, behavioural and cognitive factors are important in this process. It is these psychosocial factors, which influence individual stress perception and influence of strain on the body, which in part drive the physiological stress responses with which the model is concerned. Until now stress research in humans has tended to frame its research using a traditional transactional model. However, in order to explore the integration of emotional, behavioural and cognitive aspect of the stress response while emphasising the physiological aspects of this cumulative process the theory of allostatic load will be adopted. Up to now this theory has mainly been used to investigate animal models of stress. However, in order to advance research investigating the physiological aspects of human health across the life course, this model will be used as the basis for all four studies in this thesis.

Physiological systems typically respond to stress by initiating an adaptive response (reactivity), sustaining it until the stress ceases and then shutting the response off successfully (recovery) (McEwen & Seeman, 1999). However, it is important to

know how this single response-pattern could lead to the long term health impairments discussed above. All physiological stress mechanisms strive to maintain equilibrium, particularly during times of increased stress. This process is termed *allostasis* and refers to the body's ability to preserve 'stability through change' (Sterling & Eyer, 1988). However, under certain circumstances allostasis may be threatened, and as a result *allostatic load* is said to occur (McEwen & Stellar, 1993). For each system of the body there are both short-term adaptive actions that are protective (allostasis) and long-term effects that can be damaging (allostatic load). For example, in metabolism, adrenal steroids promote allostasis by enhancing food intake and facilitating the replenishment of energy reserves; however, over-activity of this system involving repeated HPA stimulation during stress can lead to allostatic load in terms of insulin resistance, accelerated progression towards type II diabetes, abdominal obesity, atherosclerosis, hypertension (Björntorp, 2001), reduced neuronal excitability, hippocampal atrophy and impairment of memory formation (McEwen et al, 1997a; McEwen et al, 1999). For the immune system, adrenal steroids promote allostasis by enhancing movement of immune cells to organs and tissue where they are needed to fight infection. They are also responsible for cytokine and chemokine modulation (McEwen et al, 1997b; McEwen & Seeman, 1999). With chronic over-activity of these mediators, allostatic load results, consisting of immunosuppression and an increased vulnerability to infection. Conversely, the absence of sufficient levels of glucocorticoids allows other immune mediators, such as cytokines, to over-act thus increasing the risk of autoimmune and inflammatory disorders (Sternberg, 1997).

Allostatic load may be divided into at least four sub-types, (McEwen, 1998; McEwen & Seeman, 1999; McEwen, 2000; McEwen & Wingfield, 2003a). The first type is that simply too much stress, in the form of repeated novel events, causes

recurring elevations of stress mediators over long periods of time. For example, hardship tends to be synonymous with an increase of novel, stressful events which may, predict a decline in physical and mental functioning and mortality (Lynch et al, 1997): this will be addressed further in Studies 1 and 2. A second type of allostatic load involves a failure to habituate or adapt to the same stressor. This leads to an overexposure to stress mediators because of the body's failure to eliminate the physiological stress response to a repeated event. An example of this is the fact that some individuals fail to produce habituated cortisol responses to repeated public speaking challenges (Kirschbaum et al, 1995). A third type of allostatic load concerns poor recovery from stress or failure to shut off the normal stress response; this is investigated in Studies 1 and 3. Included with this is the failure to display the normal trough of the diurnal cortisol pattern. Successful recovery is particularly important as this is when levels of catabolic hormones are reduced and energy and immune systems are restored in anticipation of forthcoming challenges. One example of this are blood pressure elevations in work-related stress that recover slowly in some individuals with a history of hypertension (Gerin & Pickering, 1995). Another example is the disruption in the diurnal cortisol pattern in depressed individuals, where chronically elevated glucocorticoid levels results in loss of bone mineral mass (Michelson et al, 1996). Finally, the fourth type of allostatic load involves an inadequate hormonal response that allows other systems, such as the inflammatory cytokines to become overactive. All four types of allostatic load are, therefore, related to long term disruption in the stress response, which is defined in terms of altered reactivity or recovery.

The main advantage of adopting the theory of allostatic load as a theoretical framework is that it uniquely attempts to explain how and why physiological responses to stress may, over time lead to ill-health. The model also attempts to investigate the

health outcomes of human stress in an integrated manner. For example, although the models focuses primarily on the physiology of the stress process (an area neglected in traditional transactional models) it still incorporates the importance of emotional, behavioural and cognitive factors in this process, in particular the model recognises the importance of individual variation when examining the stress process as a whole.

## 1.4 Individual Variation in Stress Responsivity

Although there is strong evidence for a link between stress, allostatic load and disease it is clear that those who suffer from stress-related pathology often do not have more stressful lives than those who remain healthy. Determining the psychological and physiological correlates of successful allostasis or *resilience* has become an important goal for current psychobiological investigation (Seeman & Robbins, 1994; McEwen, 2002). Four main factors are thought to affect individual stress responsivity. First, health-damaging and health-promoting behaviours that the person partakes in may vary considerably. For example, stress-related smoking can, over time, exacerbate blood pressure and atherogenesis (McEwen, 1998). Second, protective psychosocial variables are implicated as mediators of allostatic load. Individuals without these protective factors (such as social and financial support) are more vulnerable to stress and its health implications. A clear example of this is the socioeconomic gradient in relation to morbidity and mortality (Hemingway et al, 2001). Third, primary and secondary appraisals of events may also determine an individual's stress response. Two converging lines of evidence suggest that differences in past experience and personality characteristics may determine event appraisal. For example, determination of whether a situation is pleasant or threatening depends on appraisals based largely on past experience, expectations of the outcome, situational setting and previous learning (Ursin

& Eriksen, 2004). Similarly, personality factors or habitual ways of behaving might affect exposure to stress, appraisal, and choice and effectiveness of coping mechanism. For instance, low self esteem, cynical hostility, extroversion and an external locus of control have all been shown to increase individual vulnerability to stress (Lovallo, 2004). Therefore, if an individual typically appraises a situation as stressful, either through past experience or personality characteristics, physiological stress pathways are likely to be activated more frequently and allostatic load is more likely to occur. Finally, heterogeneity in physiological stress responsivity is a critical predictor of vulnerability to stress-induced pathology.

There are potentially five levels at which persons may differ in their physiological response to stress. First, in certain individuals the HACER (hypothalamic area controlling emotional responses) may be more reactive to given signals from the amygdala. That is, innate factors or experience may alter the initial responsiveness of the hypothalamus to descending activation. Second, the paraventricular nucleus may react to a greater or lesser degree to signals from the HACER. Third, the locus coeruleus may differ in the strength of signals it sends to the rest of the nervous system. Similarly, outputs to the peripheral organs via the intermediolateral cell column, the nucleus of the solitary tract, and the pituitary may differ from person to person. Fourth, individual differences may arise from the secretion of ACTH in response to CRH or the secretion of cortisol by the adrenal cortex in response to ACTH (Beuschlein et al, 2001). Similarly, there may be variation in negative feedback regulation from corticoid receptors, or differences in 11-beta hydroxysteroid dehydrogenase (11  $\beta$ -HSD) capacity to convert cortisol to cortisone. Finally, the immune system has large inter-individual differences; for example, in the activation, production and effect of cytokines (Straub et al, 2000). When investigating the relationship between stress reactivity, allostatic load

and health outcome it is this individual variation in physiological endpoints, such as cortisol, cytokine and cardiovascular function that is most commonly assessed. The four studies outlined in this thesis are therefore predominantly interested in the differential impact each individual's physiological response to stress could potentially have on their health status. Specific components of individual variability in the stress response are now considered.

### **1.4.1 Reactivity Hypothesis**

Reactivity hypothesis was initially applied to cardiovascular disease but research has now shown that cardiovascular reactivity is associated with hyper-reactivity in the SAM, HPA axis and immune system (Manuck et al, 1991; Uchino et al, 1995; Mills et al, 1995). Cardiovascular reactivity states that persistently exaggerated physiological stress responses referring to the magnitude, pattern and/or mechanism of the response can identify individuals or sub-groups with an increased risk of pathology (Lovallo & Gerin, 2003). It is thought of as a stable, largely behavioural construct (Bartels et al, 2003) and studies have shown temporal and situational stability of reactivity for various cardiovascular measures including systolic blood pressure, heart rate, heart rate variability, pre-ejection period, cardiac output, stroke volume and total peripheral resistance (Steptoe & Vögele, 1991; Nelesen et al, 1999; Hawkley et al, 2001; Riese et al, 2003; Lamine et al, 2004). It becomes possible therefore to argue that more reactive persons will be more likely to develop stress-related illness such as hypertension, high cholesterol, cardiovascular disease and memory impairment (Lovallo & Wilson, 1992b; Lovallo & Wilson, 1992a; Otte et al, 2005). Subsequently it seems logical that the tendency to hyper-respond must be stable and reproducible over time because it takes years for disease or allostatic load to develop (Gerin et al, 1994; Burleson et al, 2003).

This hypothesis is therefore an important foundation for studies 1 and 3 reported in this thesis.

### **1.4.2 Recovery Hypothesis**

Physiological response to stress not only involves reactivity to challenge but also recovery from the event. Individual levels of physiological recovery are believed to be consistent over setting and time (Rutledge et al, 2000) and there is now growing support for the notion that rate of recovery of physiological responses following a stressor is as important as the magnitude of the stress response as a marker of individual vulnerability (Hocking Schuler & O'Brien, 1997; Linden et al, 2003). McEwen (1998) has argued that delayed recovery following stress is indicative of allostatic load and may lead to breakdown in adaptation at the biological and psychological levels, although at present, work on stress-related recovery and health is limited. Similarly, this hypothesis will also be considered throughout the thesis.

## **1.5 Stress and Health in Middle and Old Age**

The prevalence of most major stress-related disorders and psychological and cognitive impairments is seen to increase substantially across the lifespan. With the ongoing and exponential growth of the world's population of older adults, the issue of ageing has become pertinent to the understanding of psychosocial contributions to disease risk (Schaie et al, 1992). Despite this, it is argued that stress research has generally been conducted in a 'life course vacuum'. Without consideration of the life course, it becomes difficult to detect the changing configurations of stressors that drive the physiological stress process (Elder et al, 1996). An important factor in this relationship may be, with advancing age, the increase in stressful life events and a

change in environmental demands (Rodin, 1986; Folkman et al, 1987). Alterations in environment can lead to increased levels of stress, which has important implications for psychological and physiological well being. In this respect, the traditional view of ageing states that elderly persons constitute a vulnerable population that are especially susceptible to increased psychological strain (Thompson & Spacapan, 1991). However, this may not be the case for all elderly individuals, and little attention has been paid to potential differences in the ability to cope with increased stress across the life course.

There are a number of ways in which the traditional view of ageing suggests that psychological stress may manifest itself in old age. First, it is contended that physical, environmental and social limitations, which typically accompany old age, may undermine one's competence and control, possibly resulting in added stress. For instance, financial power and social status are said to diminish with the onset of retirement, and these may culminate as additional stressors during old age. It is known that a wide variety of behavioural, social and psychological factors are shaped and structured by social status, but again little research has linked these outcomes to age. Those that have investigated elderly populations discovered an association between low social status, poorer physical and psychological functioning, prevalence of disability and greater need for constant care (Kubzansky et al, 1998; Melzer et al, 2000). Second, socially prevailing stereotypes about the aging process may contribute to internalizing a sense of incompetence, resulting in added stress. Third, uncontrollable adverse events and irreversible losses that cumulate in later life may also result in added psychological strain. For example, older adults who had lost a spouse showed a greater decline in memory over 6 years than those who remained married (Aartsen et al, 2005); while those with a larger social network have a longer life expectancy (Giles et al, 2005). Finally, dependency due to increased medical care may alter one's level of social stress.



For example, the onset of illness is more likely to produce depression in older than younger people (Ensel, 1991). As a result, of these factors research has demonstrated that increased stress is negatively associated with several stress-related health problems including rheumatoid arthritis (Affleck et al, 1987) and cancer (Thompson et al, 1993). Studies have also shown that a poor prognosis but lower levels of psychological strain were associated with less distress, fewer physician visits, fewer laboratory tests, fewer days of hospitalisation and better mental health for patients with cardiac complaints (Helgeson, 1992) and arthritis (Chipperfield & Greenslade, 1997). Although risks to health and environmental changes can result in added strain, there is essentially no empirical support to suggest that stress is an inescapable consequence of old age that will affect all individuals to a similar degree (Pearlin & McKean Skaiff, 1997). It would be erroneous to suggest that all functions decline with age, when in fact many abilities including intellect and wisdom, actually increase later in life. Others have also shown that as people get older they often automatically adapt their environment in order to decrease the demands it may place upon them. Subsequently, there is a need to consider the concept of successful ageing, which states that not every person will suffer from functional deterioration and that ageing can be viewed in a positive light. It appears imperative therefore, that the traditional view of ageing is questioned and that the investigation of stress and health considers the influence of personal interpretation and impact of the environment on individual populations into old age.

### **1.5.1 Stress Physiology in Middle and Old Age**

Although not every individual ages at the same rate, in general, specific areas of physiological function do tend to alter over time. These changes often have negative impacts on quality of life and can compromise independence in some individuals (Taaffe et al, 2000). An illustration of these broad alterations in physiological response

will be considered. Deterioration of the cardiovascular, neuroendocrine and immune systems has been thought to contribute to this increased morbidity and mortality associated with advanced age. In general, cross-sectional studies report little difference in basal ACTH and cortisol levels in aged healthy subjects (Seeman & Robbins, 1994; Gotthardt et al, 1995; Kudielka et al, 1999; Kudielka et al, 2000; Heffelfinger & Newcomer, 2001). However, higher evening plasma cortisol levels and lower morning basal plasma cortisol levels have been reported in some elderly samples, while phase advance in diurnal rhythm is also observed (Van Coevorden et al, 1991; Deuschle et al, 1997). Primarily though, cortisol concentrations show age-related increases during night time at the circadian trough of HPA activity (Van Cauter et al, 1996). Human ageing is also associated with complex HPA axis changes characterised by increased responsivity to central stimulation and reduced responsiveness to feedback inhibition at or above the level of the anterior pituitary. For example, a recent meta-analysis demonstrated age-related increases in cortisol response to challenge, and this response is almost three-fold stronger in women than men (Otte et al, 2005). This gender difference may be due largely to the decline of female reproductive hormones and the subsequent effect this has on cortisol production. The importance of this increased cortisol response to stress in healthy elderly subjects stems from its potential for widespread central and peripheral effects, thereby possibly increasing the risk for age-related disorders.

Similar dysregulation with age is also seen in the cardiovascular system. Between the ages of 30 and 70, decreases in the muscle mass of the heart and contractility of the myocardium produce declines in stroke volume and left ventricular wall thickness, and increases in total peripheral resistance and arterial rigidity. Additionally, maximal heart rate declines by about 24 beats per minute, contributing to

a 30 % reduction in cardiac output and a 25 % to 30 % reduction in the maximal work capacity of the heart in elderly individuals. There is also an apparent down regulation of beta-adrenergic receptors as a function of age. Although cardiac performance decreases with age, resistance to blood flow tends to increase. Not surprisingly in light of this evidence, ageing has been associated with an increase in systolic and diastolic blood pressure at rest (McNeilly & Anderson, 1996). Older persons also exhibit less heart rate variability than younger persons. This could represent either a reduction in respiratory sinus arrhythmia or baroreceptor sensitivity in elderly individuals (Simpson & Wicks, 1988). In general, both epidemiologic and laboratory studies indicate gender differences in age-related cardiovascular dysfunction. Prior to the age of 50, resting systolic and diastolic blood pressures in males is higher than in females. After the age of 60, however, females tend to show higher resting blood pressure and heart rate compared with males. Age-related increases in sympathetic nervous activity at rest appear also to be associated with an increase in morbidity and mortality in older populations; however, vulnerability to illness needs to be understood by investigating cardiovascular responses to stress in the elderly. This issue is examined in study 1.

Increases in heart rate reactivity with age have been demonstrated with psychological stressors, physical challenges and postural changes. These tend to be greater in elderly female participants and are often coupled with greater elevations of plasma catecholamine concentrations in older subjects (McAdoo et al, 1990). Similarly, systolic and diastolic blood pressure reactivity tends to be greater in elderly participants, again higher reactivity levels are seen in female participants (Steptoe et al, 1990). Lastly, there appears to be an increase in stress-related reduction of heart rate variability with advanced age.

In older persons, measures of inflammation can also be used to identify those at risk from functional decline or mortality (Reuben et al, 2002). During ageing there appears to be a loss of phagocytic capacity, decrease of oxygen free radical production, loss of natural killer cell function, decrease in T and B cell proliferation and shift in T helper lymphocyte function from Th1 to Th2 (Wilder, 1995). However, with respect to TNF $\alpha$  and IL-6, it may be that the decline of testosterone, oestrogen (Ralston et al, 1990) and dehydroepiandrosterone (DHEA) (Straub et al, 1998) leads to an increase in these cytokines, although men are reported to have greater age-related increases in IL-6 than women (Taaffe et al, 2000). An increase in proinflammatory cytokines during old age may therefore lead to muscle atrophy (Ferrucci et al, 2002), cognitive impairment (Weaver et al, 2002) and other stress-related pathology (Harris et al, 1999). However, to date the relationship between stress-related cytokine increase, age and health has not been explored. Overall, there appears to be the need to consider the study of stress physiology across the life course in order to gain a fuller picture of the relationship between environmental challenge and pathology.

### **1.5.2 Individual Stress Responsivity and Allostasis in Middle and Old Age**

When the body receives a significant challenge over many years, physiological systems may begin to operate outside the optimum range or have difficulty returning to these limits after stress. A number of studies have examined both age differences and age changes in a variety of physiological markers (reviewed in Section 1.5.2). However, there is great individual variability in rate of physiological change and its relationship with advanced age (Crimmins et al, 2003). That is, there are great individual differences in rates of human ageing and prevalence of disease or impairment

in elderly populations. This resilience to allostatic load is also known as ‘successful ageing’ (Rowe & Kahn, 1998). After increasing sharply from 20 to 60 years allostatic load is remarkably consistent in old age; therefore, individual differences in the ageing process can be conceptualised as an accumulation of wear and tear from perceived daily experiences and major life stressors which interact with the genetic constitution and early life experiences (McEwen, 2002; McEwen, 2003). For example, it has been reported that a lifetime of economic hardship can promote an earlier decline in physical, psychological, cognitive functioning and increased mortality (Lynch et al, 1997). A greater understanding of the role stress responsivity and allostatic load play in individual variability of successful ageing is therefore important. These factors are explored in Studies 1 and 2.

## **1.6 Methodological approaches to the Investigation of Stress Responsivity**

### **1.6.1 Mental Stress Testing**

Studying mediators of allostatic load in a laboratory setting have predominantly assessed individual differences in stress response. In a typical mental stress-reactivity experiment, physiological responses are recorded during an initial resting baseline period, during exposure to the stressor and during recovery from the stressor. The magnitude as well as the pattern of physiological change from resting, stress-induced and recovery levels of cardiovascular, immunological and neuroendocrine activity provides information on allostatic load and health status. A wide range of mental stress tests can be employed in this procedure, including painful stimuli, cognitive or problem

solving tasks or public speaking tasks. A recent meta-analysis revealed that combined cognitive/public speaking tasks which induce social-evaluative threat (an aspect of the self which could be judged negatively by others) or uncontrollability (a context of forced failure in which participants could not succeed despite their best efforts) reliably elicited the largest cortisol response (Dickerson & Kemeny, 2004). It seems important, therefore, that individual situation appraisal be assessed in order to gain a true reflection of task responsivity.

Measurement of neuroendocrine, immunological and cardiovascular responsivity is also an important consideration. Most studies involving blood samples use cannulation so that the added stress of needle insertion is removed. However, a number of these studies do not employ an adequate rest period, in which habituation to cannulation can occur. Alternatively the stress response may be masked by venepuncture-related anxiety where cannulation is not used. In addition, it is often not appropriate to gain blood samples when investigating very old or very young populations. Fortunately, the assessment of salivary cortisol has recently become a valuable alternative to blood-borne analysis. Since cortisol is considered to enter saliva by means independent of active transport, salivary cortisol is unaffected by salivary flow rate. Moreover, in the blood about 90 % of total cortisol present is bound to large proteins (cortisol binding protein and albumin) and the remaining 10 % is unbound. It is this unbound or 'free' cortisol, which reaches the target organs. The acinar cells lining the salivary glands prevent these proteins and protein-bound molecules from entering saliva; salivary cortisol is therefore a reliable measure of the physiologically active component of cortisol (Kirschbaum & Hellhammer, 1994).

The use of experimental procedures to measure physiological reactivity to, and recovery from an acute mental stressor permits greater standardisation of the stressor

task, stricter control over confounding variables, while allowing for direct causal inferences between stressful conditions and physiological change. This objective measure also eliminates the problems associated with self-report assessment which are subject to numerous biases (Steptoe & Vögele, 1991; Dickerson & Kemeny, 2004). This methodology will therefore be used in Studies 1 and 3.

However, despite their usefulness experimental protocols have several limitations. At present, there is no consensus on the best way to measure task recovery, and only a few studies have begun to investigate the psychometric properties of various approaches (Christenfeld et al, 2000; Rutledge et al, 2000). Even fewer studies have examined whether uncontrollable contexts that threaten the social self influence recovery processes. Furthermore, no procedure has been developed to standardise the timing of immunological samples, which makes reliable comparisons across tasks very difficult. Although the monitoring of physiological activity during cognitive and public speaking tasks goes some way toward evaluating ecologically more relevant situations the frequent use of standardised stimuli may mean that results from some investigations are somewhat divorced from everyday life. Finally, a wide range of factors is known to influence cardiovascular and neuroendocrine stress-responses, including aerobic fitness, medication use, physiological or psychological disorders, diet and alcohol and cigarette intake; however, some studies fail to include statistical control for these variables. As a result, it is believed that mental stress testing should be used as one of a range of strategies through which to investigate stress reactivity and allostatic load. This thesis will therefore adopt a multi-methodological approach in order to assess various aspects of allostatic load from a more holistic perspective.

### **1.6.2 Clinical and Naturalistic studies**

Laboratory based experiments are an important means of assessing individual stress-responsivity; however, they may not be applicable to certain populations, particularly the elderly. For example, the alien environment may induce unreliably high levels of stress confounding baseline measures. Similarly the employment of difficult or unfamiliar tasks with an elderly sample may cause undue distress or added confusion leading to possible ceiling effects. For elderly populations, naturalist studies are recommended for the assessment of stress-related allostatic load. Naturalistic studies are carried out in the person's everyday environment, and three types are relevant to the psychophysiological aspects of health. The first involves assessment during challenging tasks within the natural environment. These investigations are most similar to laboratory studies in that they evaluate responses to acute and relatively unusual events, therefore maintaining an element of extraneous variable control. The second type of study involves the use of ambulatory apparatus to measure physiological activity in a relatively unobtrusive fashion throughout the course of the day. For example, ambulatory blood pressure monitors and electrocardiograms have been valuable for assessing psychosocial factors related to cardiovascular disease (Pickering, 1991; Horsten et al, 1999). The third type of study involves repeated assessment of physiological parameters in the natural setting. For instance, repeated samples of cortisol can be obtained over the day and analysed to measure the profile of cortisol release in people exposed to different levels of daily stress (Steptoe et al, 2003a; Kunz-Ebrecht et al, 2004a; Kunz-Ebrecht et al, 2004b).

The main advantage of naturalistic studies is their high level of ecological validity. It is argued that in studies of blood pressure of patients with hypertension, ambulatory measures are even more predictive of pathological progression than



measures taken in the clinic (Pickering, 1991). There are, however, several limiting factors which need to be taken into account. First, naturalistic studies are inevitably somewhat disruptive; subsequently people are aware of the unusual apparatus and may modify their behaviour accordingly (Blanchard et al, 1990). Second, there are numerous extrinsic factors which need to be taken into account, including cigarette smoking, food and caffeine intake, and patterns of sleep and physical activity. Perhaps most problematic is distinguishing physiological responses related to physical activity from those elicited by psychosocial factors (Van Doornen et al, 1994). Finally, research in the area of cardiovascular reactivity suggests that the link between reactivity in the laboratory and reactivity in the natural environment may be weak (Gerin et al, 1998), while research comparing cortisol response in the laboratory and in the naturalistic setting is limited (Dickerson & Kemeny, 2004); although this may not necessarily be a problem of reliability but rather a difference in the nature of the two stress situations. Future work is recommended to combine laboratory and naturalistic studies in order to gain a more holistic view of stress responsivity and health across the life course. As a result this naturalistic methodology will be employed in Study 2.

### **1.6.3 Intervention Studies: Experimental Manipulation of Physiological Processes**

The two approaches outlined so far have various merits especially when collecting data from an elderly sample. However, both these methodologies do not allow for the exact causal nature of the relationship between stress responsivity and health to be established. Laboratory studies using mental stress tests (such as those carried out in Study 2 and 3) can be considered ‘quasi-experimental’ or descriptive because group allocation of individuals (such as high and low responders) can only be

done once the challenge has been administered. In contrast, ‘true experiments’ are defined by manipulation of the independent variable so that the exact effect of a specific factor on health (a physiological process in this case) can be assessed. As a result, the causal direction of the relationship can be ascertained. Intervention studies are crucial when trying to determine the possibility of a bi-directional relationship between behaviour, the central nervous system and immunological regulation; this process is adopted in Study 4.

Intervention studies also benefit from high internal reliability. However, this can only be achieved if certain factors are taken into consideration. First, a true experiment must ensure that all variables (besides the manipulated independent variable) are controlled. The laboratory setting usually allows all random variables and constant errors to be eliminated by standardising conditions. Second, allocation of participants to experimental condition must be random; this ensures that any possible individual differences in each group will be evened out. Finally, to guarantee high internal validity the intervention must reduce participant demand characteristics and experimenter effects which could bias the study’s outcome. To achieve this, two experimental designs can be adopted simultaneously. The first research design involves the use of a placebo-control group. For example, when manipulating physiological stress mechanisms, pharmacological or vaccine stimuli are often used. In order to investigate the effects of this artificial stimulation a control group will often be given an inert substance that is identical in appearance to the active one. Second, placebo-control techniques can also be used in combination with a double-blind design. Here both experimenter and participant are unaware of what substance is being administered. In this way the effect of physiological processes on behaviour can be assessed without the

participant altering their response in accordance with their beliefs about which group they are in.

Laboratory interventions are usually criticised for low construct validity, artificiality and their inability to generalise to real life situations. However, in the study of physiological mechanisms, such as those investigated in Study 4, the experimental setting becomes less important as the response is manipulated so that it is similar to one that would be observed in a naturalistic setting. Similarly, the experimental setting is often the only place that such mechanisms can be accurately explored, and this procedure also allows for future replication.

#### **1.6.4 Section Summary**

This section has outlined the advantages and disadvantages of studying stress responsivity using a variety of methods. What has become clear from this is that no single procedure is satisfactory for investigating all aspects of physiological responsivity and health across the life course. As a result the thesis will employ a variety of complimentary procedures and design from which to gain a fuller, more integrated picture of individual differences in response to allostatic load.

### **1.7 Aims**

This dissertation describes a series of four studies exploring the effect of individual differences in neuroendocrine, cardiovascular, immunological stress responsivity and health. The concept of allostasis and physiological responsivity is immense, making it impossible to cover every aspect of these topics in detail. The thesis will aim therefore to examine various domains of the physiological responsivity hypothesis, within a variety of populations and health outcomes which are currently

under investigated or inconsistently reported. The importance of studying individual vulnerability to allostatic load from a life course perspective will also be highlighted. Specifically, this work will endeavour

- 1) To investigate the association between individual differences in physiological reactivity and recovery from challenge and cognitive performance in an elderly population
- 2) To investigate the association between cortisol awakening response dysregulation, social status and allostatic load in elderly individuals
- 3) To explore whether physiological stress-responsivity precedes the development of cardiovascular disease in young adults most at risk
- 4) To examine the bi-directional nature of the relationship between stress, immunological and neuroendocrine responsivity and psychological health

The thesis also aims to emphasise the importance of adopting a multi-methodological approach to studying stress responsivity across the life course. The analytic strategy therefore involves; first, cross-sectional comparisons of elderly response to challenge in a health care setting; second, an examination of social status differences in cortisol awakening response from the same elderly sample in a naturalistic environment; third, the administration of an acute mental stressor to young adults with and without risk of cardiovascular disease; and finally the use of a double-blind placebo-control intervention to investigate the bi-directional nature of stress, immunological function and psychological wellbeing. These strategies will help to further understanding of certain aspects of the physiological stress responsivity

hypotheses which may contribute to individual allostatic load and subsequent health impairment.

## **Chapter 2: Physiological Correlates of Cognitive Functioning in an Elderly Population (Study 1)**

### **2.1 Introduction**

Individual responsivity to challenge is believed to affect cognitive health over the life course. As a result cognitive decline is sometimes viewed as an inevitable consequence of old age. However, ageing is a multifactorial process that results in heterogeneous patterns of morbidity, implying that individuals will not display the same rates of physical, mental or cognitive decline (Meaney et al, 1995). The complex process of ageing is thought to be influenced by external stimuli such as level of education, social networks and fitness status, which if adverse may go on to alter the body's internal control systems (Arbuckle et al, 1992). Two of the best characterised homeostatic response systems are the hypothalamic-pituitary-adrenal axis and the autonomic nervous system which co-ordinate multiple neuroendocrine, metabolic and cardiovascular responses. The importance of age-related change in patterns of neuroendocrine and cardiovascular response to challenge stems from their potential to influence mental, physical and in particular cognitive functioning. Interest in possible age-related changes in allostatic regulation has been stimulated by the fact that individuals over 65 now represent the fastest growing section of the population. Estimates for the United States alone project that by the year 2050 more than one in five people will be aged 65 and over (Otte et al, 2005). With these recent societal changes the investigation of mild cognitive impairment, which has a serious impact on social and

occupational achievements, quality of life and health, family and care resources has become increasingly important (Launer et al, 1995; Waldstein, 2003).

Mild cognitive impairment refers to the transitional stage between normal ageing and dementia (Petersen et al, 2001), reflecting, in particular, evidence of memory decline in the absence of clinical dysfunction (Rapp et al, 2002). Mild cognitive impairment is important in terms of recognising memory loss in older people as well as identifying those at increased risk from Alzheimer's disease and those who could benefit from preventative strategies (Burns & Zaudig, 2002). Those with mild cognitive impairment have a 50 % increased chance of developing Alzheimer's disease within a two year period (Bozoki et al, 2001). However, like other aspects of ageing the occurrence of mild cognitive impairment and age-related memory decline is not inevitable. The heterogeneity in patterns of cognitive ageing remains incompletely understood, suggesting a need for the examination of additional factors that may contribute to the observed differentiation in patterns of cognitive decline. Subsequently, attention has focused on the involvement of the neuroendocrine and cardiovascular factors in this process. In particular, elevated cortisol, blood pressure and heart rate activity are hypothesised to contribute to hippocampal damage and memory decline (Lupien et al, 1998; Petrovitch et al, 2000; Lathe, 2001).

### **2.1.1 Neuroendocrine Dysregulation and Cognitive Performance**

Today there is evidence from clinical and laboratory studies to suggest that an inverted 'U-shaped' relationship exists between HPA axis dysfunction and cognitive performance (Lupien & McEwen, 1997; De Kloet et al, 1999). In particular, excess

availability of glucocorticoids have been found to negatively effect human cognitive function, especially explicit<sup>1</sup>, declarative memory<sup>2</sup> (McEwen et al, 1999).

When investigating the relationship between cortisol and memory three main methodological designs are employed. The first explores the cognitive performance of a population with raised basal cortisol levels; these raised levels are mainly due to the presence of disease or old age. The second examines the relationship between memory and stress-related cortisol elevation by assessing cognitive performance after a laboratory-based stress-inducing challenge, and the third investigates the effect of pharmacologically manipulated cortisol levels on memory performance. Each of these approaches will be considered in turn.

First, the relationship between cortisol and memory is often explored by studying populations with elevated basal cortisol levels. The majority of these investigate disease-related cortisol elevations. For example, patients with Cushing's disease, an endocrine disorder characterised by corticosteroid over-production, show significant positive correlations between hippocampal formation volume and verbal declarative memory scores, and significant negative correlations between hippocampal formation volume and plasma cortisol levels (Whelan et al, 1980; Starkman & Schteingart, 1981; Starkman et al, 1986; Starkman et al, 1992; Mauri et al, 1993). In addition, depressed patients with evidence of pituitary-adrenal dysinhibition and consequent hypercortisolaemia demonstrate impaired explicit memory (Wolkowitz et al, 1990). Moreover, patients with Alzheimer's-related cortisol hyperactivity have been shown to have an inverse relationship between 24 hour cortisol levels, severity of cognitive decline and hippocampal atrophy (De Leon et al, 1988; Martignoi et al, 1990; O'Brien et al, 1996; Hartmann et al, 1997; Oxenkrug et al, 1999). Although, such

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<sup>1</sup> Conscious or voluntary recollection of previous information (Lupien et al, 1994).

<sup>2</sup> Hippocampal-mediated memory responsible for the recollection of facts and events (Schacter, 1997).



studies of endogenous disorders do appear to suggest that glucocorticoid dysregulation can impair memory, this research generally fails to discriminate the cognitive deficits related to cortisol hyperactivity from those associated with the underlying illness itself; although basal levels of salivary cortisol have been related to spatial memory in healthy young volunteers (Van Honk et al, 2003). Furthermore, interpretation of results is often limited by the nonrandomised treatment assignments, non-causal associations and the failure to account for alterations in memory function that are attributed to acute or long term disease-related changes in the brain which do not involve direct glucocorticoid effects (Heffelfinger & Newcomer, 2001).

In addition to disease-related cortisol elevation, the relationship between raised basal cortisol levels and memory are often studied in elderly populations. These elevated cortisol levels observed in elderly participants may result in cognitive impairment and hippocampal atrophy (Seeman et al, 1997a; Lupien et al, 1998; Lupien et al, 2005). For example, men and women over 70 with low basal cortisol levels performed significantly better than those with higher basal cortisol levels on several tests of declarative memory (Carlson & Sherwin, 1999). Longitudinal data from healthy, cognitively intact, volunteers (aged 60-87 years) showed that increases in 24 hour cortisol activity were correlated with poorer memory performance in the fourth year of the study (Lupien et al, 1994). In contrast, two studies reported that low cortisol levels were associated with superior memory function, but only in young males and post menopausal women; no relationship was found between cognition and cortisol in the elderly comparisons (Seeman et al, 1997a; Wolf et al, 2001b). A summary of the available evidence therefore suggests that ageing *per se* is probably not associated with universal, uniform declines in cortisol and cognitive function but rather with differential patterns of cortisol-related ageing individual to each person.

Second, the relationship between neuroendocrine activity and memory may also be examined by studying cortisol release as an indicator of increased arousal. For instance, it is proposed that elevated cortisol levels can be stimulated by acute laboratory stress and used as an indicator of neuroendocrine activity. Thus, studies using psychological stressors have found that participants who show a pronounced cortisol increase to a stressor tend to make more errors on declarative memory tasks (Kirschbaum et al, 1996; Lupien et al, 1997; Wolf et al, 2001b) and working memory tasks (Al' Absi et al, 2002) than subjects showing a mild cortisol response (Al' Absi et al, 1998). However, such studies do not control for the difference in memory scores brought about by a stress-induced increase in concentration and attention.

Finally, it is common when studying memory and glucocorticoids to manipulate cortisol levels pharmacologically in order to exert a higher degree of experimental control. In a comprehensive study measuring verbal declarative memory performance, with controls for attention, arousal and visuospatial function, a rising dose of the natural glucocorticoid hydrocortisone given to healthy volunteers over a 4 day period, produced impairments in verbal declarative memory but had no effects on other areas of cognition (Newcomer et al, 1999). By administering hydrocortisone at varying times to different groups of healthy volunteers (one hour before word presentation to test acquisition of memory; immediately after word presentation to test consolidation; and one hour before delayed recall to test retrieval), De Quervain (2000) also found that only declarative memory retrieval was affected by acute exposure to glucocorticoids: there was no effect on memory acquisition or consolidation. Similarly, Wolf et al (2001a) discovered that cortisol administration impaired recall of word lists learned before drug administration in both young and elderly men but that cortisol had no effect on word learning or delayed recall learned after cortisol administration. However, results are conflicting as

two major studies with healthy volunteers observed no or mixed results when cortisol-treated subjects were tested in an immediate word recall task (Fehm-Wolfsdorf et al, 1993; Porter et al, 2002; Tops et al, 2003). A recent meta-analysis revealed that inconsistencies in the literature may be due to the timing of cortisol administration (Hett et al, 2005). The authors concluded that studies which administered cortisol before learning, on average had no effect; however, studies which administered cortisol before retrieval reported a significant decrease in memory performance.

Most studies of drug-manipulated cortisol effects on human memory function have been limited to brief (60 minute), single dose (10-50 mg) treatments (Beckwith et al, 1986; Fehm-Wolfsdorf et al, 1993; Kirschbaum et al, 1996). As a consequence, these study designs do not model the longer duration of hypercortisolaemia relevant to most stress-related human events, thus preventing an assessment of memory performance over the time course expected for genomic glucocorticoid actions. It is unclear how reliable pharmacologically manipulated cortisol levels are when assessing memory decline. However, impairment of memory during glucocorticoid treatment of healthy subjects does occur during suppression of CRH and ACTH. These studies provide useful evidence that increased glucocorticoid levels, rather than changes in other cognitively active components such as adrenaline, noradrenaline, ACTH and CRH, are most likely responsible for memory effects.

In summary, the literature presented appears to suggest some kind of detrimental relationship between cortisol and memory. However, this evidence is contradictory. It is proposed that these differences may result from the failure to account for individual variability in cortisol response. Such a suggestion questions studies which look at set cortisol levels, that is, basal or artificially induced levels. It is proposed that it is one's

reaction to a challenging situation (cortisol responsivity level) and not the presence of stress itself which is essentially responsible for memory decline.

### **2.1.2 Cortisol Responsivity and Cognitive Performance**

When one is faced with a challenging situation, the hormones that are released during the period of activation maintain the body's equilibrium by means of allostasis. However, if activation of the HPA axis is frequent, and allostasis is extended over time, allostatic load results. This can have detrimental effects on both physical and cognitive health (McEwen & Stellar, 1993). Allostatic state can be gauged by measuring the response profile of primary mediators (such as cortisol) involved in the stress response. Therefore, if a person were frequently to interpret a situation as stressful, that is, if they frequently reacted strongly to stress (known as high-reactors) then allostatic load could occur. Alternatively, prolonged activation to a stressor, that is where physiological mediators are not shut-off effectively once the stressor has ceased (known as poor-recovery), could also result in allostatic load (McEwen, 2002). In both cases the individual will be producing high levels of cortisol. Accumulated exposure to glucocorticoids can then go on to compromise hippocampal integrity (Lupien et al, 2002). Hippocampal damage can trigger a positive-feedback loop of continually increasing cortisol levels, subsequently resulting in more hippocampal disturbance and greater memory decline (Porter & Landfield, 2004). It is suggested that individual differences found in response patterns (either through reactivity or recovery) may explain the variability found in successful ageing. Investigations which examine individual variability have therefore produced more definitive results.

In a study of 58 healthy young subjects Wolf et al (2001b) discovered that individuals exposed to a psychological stressor (Trier Social Stress Test) showed no impairment of declarative memory performance in comparison to the control group.

However, performance on the same word recall task with a different sample was impaired for those participants who had an elevated cortisol response to the stressor. This correlation was solely caused by the strong associations observed in men (Kirschbaum et al, 1996). Similarly, Kirschbaum, et al (1996) reported that 13 young participants demonstrated a significant negative relationship between stress-induced cortisol levels and performance on a declarative noun identification and recollection task but only in those subjects regarded as ‘high cortisol responders’. Lastly Lupien et al (1997) reported that in a study of 14 healthy elderly participants a stress condition significantly reduced pair associated word recall in comparison to those in the control group. They also discovered that some subjects, regardless of group allocation produced an elevated cortisol reaction 60 minutes prior to the task in comparison to the other subjects who produced elevated cortisol levels just 25 minutes before the task. These two groups of individuals were labelled as ‘cortisol responders’ and ‘cortisol non-responders’, respectively. It was discovered that cortisol responders had greater memory impairment after completion of the task than cortisol non-responders. This suggests that individual anticipation of a stressor and cortisol reaction to the challenge is more important in influencing cognition than the existence of the stressor itself. However, one study reports conflicting results which suggests a need for strict methodological considerations in future research.

Domes et al (2002) discovered that ‘high cortisol responders’ exposed to a stressor demonstrated *increased* memory performance in comparison to low cortisol responders who demonstrated poorer word retrieval. On the authors’ own admission they accept that the conflicting results may be due to a string of methodological limitations which need to be addressed in similar future investigations. First, the memory test employed may have been less sensitive to detect stress-induced memory

deficits, suggesting the need for a more ecologically valid or engaging stressor to be used. Second, sample characteristics such as gender may affect the relationship between glucocorticoids and memory. Only female participants were tested in this study and previous research has shown greater cortisol effects for men. Lastly, the authors speculate that stress may have enhanced memory either due to the dual activation of the autonomic nervous system as well as the HPA axis, or by the effects of stress-induced motivation and intentional processes. For example, it is argued that both noradrenaline and adrenaline may have memory enhancing effects which could override the effects of cortisol (Liang et al, 1986; Introini-Collison & McGaugh, 1986). It therefore seems important to examine the impact of both the ANS and HPA axis and memory function at the same time. More likely however, is the case that non-specific motivational and attentional processes modulate the relationship between effort arousal and memory. For instance, subjective task appraisals or a motivation to perform well could raise cortisol levels whilst also altering task performance independently of physiological reactivity to the tasks themselves. This suggests that in future, participants should rate their levels of involvement, anxiety, difficulty and attention during cognitive tests in order to control for influence of these factors on cognitive performance.

Despite these limitations the literature seems to suggest that increased cortisol is related to reduced memory performance, but more importantly that not all individuals experience the same degree of neuroendocrine activation (Sauro et al, 2003). Therefore, those people who consistently produce elevated cortisol levels when faced with a challenging situation may be at higher risk of cognitive dysfunction and subsequent dementia in older age. In addition, it must be considered that when one experiences a stressful situation, the body's autonomic nervous system is also activated. If

information is perceived as threatening emotional responses are generated in the limbic system. The hypothalamus then controls both the HPA axis and sympathetic adrenal medullary (SAM) response system which is responsible for catecholamine, heart rate and blood pressure increases. The autonomic and endocrine systems are functionally distinct, but they act together to regulate and co-ordinate the body's stress response. It is important therefore, to investigate the impact of both cardiovascular and cortisol responses on memory function.

### **2.1.3 Blood Pressure Responsivity and Cognitive Performance**

Elevated blood pressure is an established risk factor for stroke, coronary artery disease, kidney and eye damage (Waldstein, 2003). It also contributes to silent small vessel disease and white-matter hyperintensities (Van Swieten et al, 1991). On magnetic resonance imaging scans white-matter hyperintensities appear as bright areas reflecting local increases in brain water content (Snöderlund et al, 2003). In turn, these cerebral lesions have been associated with reduced memory performance. Because blood pressure elevation is increasingly common as people age the exploration of blood pressure and cognition is pertinent to the study of mild cognitive impairment in 'normal ageing'.

The relationship between elevated blood pressure and memory is typically studied by employing three different methodologies. The first involves comparison of populations with certain blood pressure-related disorders to those with normal blood pressure. The second uses longitudinal studies to examine the ability of basal blood pressure to affect future memory decline. And the third examines the relationship between memory and blood pressure responsivity in healthy populations using a correlational design.

First, elevated blood pressure and memory impairment are frequently studied by comparing patient populations to those with normal blood pressure. For example, hypertension, diabetes mellitus, myocardial infarction and low levels of physical fitness have all been associated with impaired cognitive function (for reviews see Dustman et al, 1990; Waldstein & Elias, 2001). Farmer et al (1990) discovered that medication-free hypertensives demonstrated a significant inverse relationship between blood pressure and cognitive performance. However, they concluded from a review of the literature that this relationship is inconsistent, as studies display positive, mixed or no relationship between blood pressure and memory. Waldstein et al (1996) found that young hypertensive men (23-40 years) scored significantly worse than normotensive men on memory tasks but no such relationship existed among middle aged subjects (41-56 years).

Second, studies have also examined the relationship between blood pressure and changes in cognitive function over time. For example, participants in the Framingham Heart Study with persistent blood pressure elevation (measured over a 10 year period) had poorer cognitive function (including declarative memory) 12-14 years later, when compared with those with lower blood pressure levels (Elias et al, 1993). Similarly, analysis from the Western Collaborative Group Study found that elevation of both diastolic and systolic blood pressure during middle age predicted cognitive impairment 25 years later (Swan et al, 1996).

Lastly, studies have assessed the relationship between memory performance and stress-related blood pressure taken during the cognitive test. Results of these studies generally indicate that increases in blood pressure are associated with incremental reductions in cognitive function. For example, Pandav et al (2003) discovered that there was a significant inverse association between memory function and systolic blood



pressure taken during the cognitive test. Similarly, Budge et al (2002) found that for every 30 mmHg rise in systolic blood pressure the Cambridge Examination for Mental Disorders in the Elderly score decreased by one point. Tzourio et al (1999) stated that this relationship persisted even at 4 year follow-up. In contrast others have found those with higher diastolic, but not systolic blood pressure had a significantly lower performance on a memory task (Wallace et al, 1985; Izquierdo-Porrera & Waldstein, 2002). For instance, diastolic, but not systolic blood pressure in 1106 subjects aged 65-95 years was associated with memory impairment in subjects 75 years and over (Cacciatore et al, 1997). However, results of such experiments are not consistent, with at least two studies reporting no associations between systolic and diastolic blood pressure and memory performance (Van Boxtel et al, 1997; Glynn et al, 1999).

In summary, there appears to be no consistent relationship between blood pressure and memory test performance. It may be the case that a negative relationship between blood pressure and memory does exist but that this only occurs in those individuals who present exaggerated reactions and/or prolonged recovery to cardiovascular arousal. The memory of individuals who exhibit enhanced physiological response to cognitive testing creating a persistent state of over-arousal, may become impaired due to allostatic load. It is this individual response to challenge and its relationship to cognition which needs further exploration.

#### **2.1.4 Heart Rate and Memory Impairment**

During the stress response heart rate is also known to increase. Indices of heart rate and heart rate variability provide an insight into autonomic modulation of the heart. In population studies decreased heart rate variability has predictive value for mortality among healthy adults, and is a well established risk factor for dysrhythmic events, cardiovascular disease, sudden cardiac death caused by a decrease in baroreceptor

modulation of the heart (Simpson & Wicks, 1988). Heart rate variability is often significantly lower in elderly populations (Jensen-Urstad et al, 1997) and like blood pressure the use of this physiological measure has been related to underlying autonomic activity and individual differences found in cognitive tasks (Bucks & Ryan, 1992). However, to date very few studies have looked specifically at the effect on memory performance. Those that have, for example Faucheux et al (1983), suggest that certain individuals do have longer heart rate recovery periods than others, when faced with a cognitive challenge, but these studies typically fail to relate heart rate to the scores of the memory task itself. Those that do investigate the relationship between heart rate variability and cognitive performance seem to indicate an association between heart rate variability reactivity to challenge and memory performance. For instance, Bucks & Seljos (1994) found that as memory load increased, good performers had a smaller heart rate variability decrease, while poor performers had a large heart rate period variability decrease. Similarly, Hansen et al (2003) discovered that the high heart rate variability group performed better on two separate cognitive tasks. However, these tasks were tapping working memory and executive function, before a very short (5 minute) recovery period was analysed. Heart rate variability does appear therefore to be related to cognitive performance but the work in this area is sparse and vague. Similarly, research has not addressed the relationship between heart rate responsivity and cognitive performance. Future investigation needs to concentrate on determining exactly which area of cognition, if any, is associated with heart rate reactivity and / or recovery to challenge and in turn whether individual variability exist within these responses.

### 2.1.5 Summary and Methodological Critique

Neuroendocrine and cardiovascular dysregulation does appear to be associated with memory performance, but only in those individuals who react strongly to certain challenging situations. So far evidence is inconsistent and methodological restrictions may explain the relationship.

First, several of the studies reviewed were conducted on young or middle-aged adults (Bucks & Seljos, 1994; Kirschbaum et al, 1996; Wolf et al, 2001b; Domes et al, 2002; Buss et al, 2004) and so their implications for memory function in old age are not clear. Other studies involved rather small sample sizes. For example, the investigations reported by Lupien et al (1997; 1998a), Seeman et al (2001), Porter et al (2002), all employed fewer than 20 subjects. The power of the studies for detecting associations between physiological dysregulation and memory is therefore restricted.

Second, a number of studies were conducted with participants of a single gender (Fauchaux et al, 1983; Wolf et al, 2001a; Domes et al, 2002; Hansen et al, 2003), while those that did include male and female volunteers did not analyse the effects of cortisol, blood pressure and heart rate on memory separately for both groups (Van Boxtel et al, 1997; Glynn et al, 1999; Tzourio et al, 1999; Budge et al, 2002; Pandav et al, 2003). This may again restrict the validity of results as the relationships between physiology and memory has been shown to differ for men and women (Cacciatore et al, 1997; Seeman et al, 1997a; Seeman et al, 2001; Wolf et al, 2001b).

Third, research on older samples is often carried out in neurological units where participants have advanced cognitive disorders and are likely to complain of memory deficits. Such findings may not be relevant to the older population in general. More ecologically valid settings should be considered especially as it is estimated that 50 % of

the population over 65 present some symptoms of mild cognitive impairment in general practice (Artero & Ritchie, 2003).

Fourth, several confounding factors which affect blood pressure, heart rate, cortisol and memory performance are often not controlled or taken into account statistically. These include age, education, medication use, time of day, and evidence of other chronic illnesses (Waldstein et al, 1991; Lupien & McEwen, 1997). Education is particularly important, since those with higher educational attainment are likely to show superior cognitive performance (Stewart et al, 2003). In addition, an individual's appraisal of their task performance can affect test outcome, as well as exerting an independent effect on cardiovascular and neuroendocrine activity. Research has demonstrated that age-related variation in task interpretation (Manuck et al, 1991), social-emotional goals (Carstensen & Freund, 1994), self esteem (Albert et al, 1995; Seeman et al, 1995; Seeman et al, 1996) and motivation (Hess et al, 2001) may account for age differences in memory performance. Kelly et al (1996) also found that ratings of task involvement, task difficulty and self efficacy were all predictive of memory performance scores. It may be that a positive or negative appraisal of the task could increase physiological reactivity as well as independently increasing memory performance. Consequently, this would mask any direct affect that task-induced physiological responsivity may have on cognitive functioning. Although the mechanisms mediating this effect are not entirely clear, Steele (1997) has speculated that 'stereotype threat' may affect the cognitive performance of older individuals by raising anxiety levels and lowering motivation, both of which may result in poor performance (Levy, 1996) and altered physiological arousal (Levy et al, 2000). Hess et al (2003) discovered that when participants were told that the study would be examining ageing-related memory decrement, their judgements about negative trait terms were

faster and those about positive traits were slower following brief exposure to the word ‘old’ than they were when they were told that the study was examining positive aspects of ageing. Furthermore, younger adults, for whom ageing stereotypes were not applicable, did not exhibit significant variation in recall across experimental conditions. Because task appraisal may be responsible for elevated physiological responsivity, as well as increases in cognitive performance, it is imperative that these factors are taken into account when examining the association between stress responsivity and cognitive function in an elderly sample. Without controlling for subjective tasks appraisals it will remain unclear whether task-induced physiological reactivity can also assert an effect on cognitive performance independent of these psychological factors.

Fifth, the association between stress and memory impairment may be affected if invasive techniques are employed, particularly for the collection of blood and urine (Lupien et al, 1997). Salivary cortisol samples are therefore recommended for use with elderly participants in order to reduce the number of confounds on memory performance.

Sixth, the selection of the memory task should involve careful consideration. For instance, some studies used tasks that may not be of sufficient sensitivity to demonstrate the differences between two conditions (Glynn et al, 1999; Wolf et al, 2001b; Porter et al, 2002; Domes et al, 2002). Others used only a single test of declarative memory, while many have failed to measure memory functions on their own, instead using tasks which tested a compilation of individual cognitive functions such as intelligence, general knowledge and memory (Cacciatore et al, 1997; Tzourio et al, 1999; Budge et al, 2002).

Lastly, and perhaps most importantly, studies investigating the effect of stress responsivity on memory performance have generally been separate from those assessing

cardiovascular stress responses. Although one recent study compared pharmacological manipulation of adrenocortical and  $\beta$ -adrenergic pathways in young men (Maheu et al, 2004) to our knowledge no existing investigation has examined the effects of blood pressure, heart rate and cortisol on memory performance in one single study. Results from Maheu et al (2004) showed that propranolol ( $\beta$  receptor antagonist), but not metyrapone (cortisol synthesis inhibitor) impaired declarative memory for emotionally arousing material, thus demonstrating that adrenergic and corticosteroid hormonal systems differentially affect the declarative memory process. However, it is not yet known whether the effects of the cardiovascular system on memory performance are independent or complementary to the effects of the neuroendocrine system.

### **2.1.6 Investigation Aims**

The present investigation was designed to address several of these methodological limitations. Although the study was cross-sectional, it involved a sample of older men and women drawn from the community, and recruited through general practice. The study controlled for various confounding variables including age, medication, chronic illness and educational attainment, and utilizes non-invasive physiological monitoring procedures. The large sample of male and female elderly participants were administered two declarative memory tasks and one test of perceptual organisation and fluid reasoning ability, cortisol and cardiovascular variables were monitored before, during and after tasks. This contrast of tasks allowed one to test whether hippocampal-related declarative memory function (instead of non-hippocampal-driven functioning) was associated with individual variability in cortisol, heart rate and blood pressure reactivity and recovery.

### **2.1.7 Hypotheses**

It was hypothesised that

- Neuroendocrine and cardiovascular responsivity to a cognitive challenge would be significantly negatively associated with declarative memory performance.
- There would be no significant association between cognitive reasoning skills and neuroendocrine or cardiovascular response to challenge.
- An association between physiological responsivity and task performance would be independent of subjective task appraisals.

## **2.2 Method**

Please note that this study has recently been published in

*Psychoneuroendocrinology* (reprints can be found at the end of the thesis).

### **2.2.1 Design**

The investigation adopted a cross-sectional observational design across two sites. To explore the relationship between physiological responsivity and cognitive function in an aged population a series of standardised questionnaires and cognitive tests were administered. The extent to which this relationship is mediated by cardiovascular and neuroendocrine factors was also explored using a number of physiological measures.

### **2.2.2 Participants**

The current investigation took place as part of a larger research study on psychobiological aspects of healthy functioning on old age. One hundred and thirty nine participants aged 65-80 years were recruited by letter from two GP practices in the

London area (one low socioeconomic area and one high socioeconomic area) (Appendix I). The patient databases were searched for men and women aged between 65 and 80 who were dwelling in the community, and had no record of coronary heart disease, tachycardia, aortic valve regurgitation, dementia, psychosis, mental illness (including anxiety and depressive disorders) and no cancer evident in the last five years; these factors can affect the accuracy of cardiovascular monitoring, cognitive testing or mimic hormonal changes seen during the stress response. Names were selected at random from these screened lists, and individuals were invited to participate in a study of ageing and health (see Appendix II for participant information sheet). A total of 634 invitations were distributed, and 161 patients agreed to participate (25.4 %). 145 patients actively declined to participate and 21 invitations were returned as incorrectly addressed. There was no difference in response rate from the two different areas. It is not known how many of the remainder received the invitations but decided not to take part without informing us, and how many had moved house, died, or had medical conditions that precluded taking part that were not on the health centre records. Data were incomplete for six of the 139 participants, so analyses were carried out on 133 individuals, 59 male (42 %) and 80 female (58 %) with a mean age of 70.5 years ( $\pm 4.0$ ). On the day of the appointment all participants felt well and refrained from drinking tea or coffee, or eating a heavy meal one hour before their appointment. Informed consent was gained from each participant and ethical approval was granted by Camden and Islington Local Research Ethics Committee (Appendix III).

### **2.2.3 Cognitive Tasks**

Cognitive performance was assessed with two memory and one reasoning task selected from the third editions of the Wechsler Adult Intelligence Scale (WAIS-III) and Wechsler Memory Scale (WMS-III). These scales are among the most widely used,



non-timed tests of intelligence, cognitive ability and memory in populations aged 16-89 (Tulsky & Ledbetter, 2000). The various subscales have proved sensitive enough to detect subtle cognitive deficits related to glucocorticoid imbalances (Lupien & McEwen, 1997) and to have excellent psychometric properties (Iverson, 2001; Tulsky & Haaland, 2001).

#### **2.2.3.1 Memory Task: Verbal Paired Associates (VPA-WMS-III)**

The Verbal Paired Associates test is widely used and primarily taps declarative, episodic memory. During the task the participant was read a list of eight unrelated word pairs (Appendix IV). The first word of each pair was then repeated, and the participant was asked to supply its associated pair. This procedure was repeated three times with the words presented in a different order each time. The test was scored by summing correct responses across all four trials to produce the Verbal Paired Associates 1 (VPA 1) performance score. Results could range from 0-32, with 32 representing the best possible memory performance (see Appendix V for instructions).

Following a 30 minute interval, during which the reasoning task was carried out, participants were given the first word from each pair presented in VPA 1 and asked to recall the matching word. The total number of word pairs recalled constituted the score for the Verbal Paired Associates 2 (VPA 2) task, with values ranging from 1-8 (with 8 representing the best possible recall performance) (see Appendix VI for instructions).

The VPA-WMS-III manual details extensive standardised norms obtained from a partially stratified American sample of 1250 healthy adults aged between 16 & 89 years. This allowed age equivalence scaled scores to be determined (Litchenberger et al, 2002). These scaled scores were used in the analysis and meant that reliable comparisons with other age groups and samples could be made.

### **2.2.3.2 Reasoning Task: Matrix Reasoning (MR-WAIS-III)**

The second task was Matrix Reasoning, a non-verbal analogy-like test newly designed for the third edition of the WAIS. The test which tapped perceptual organising abilities and fluid reasoning is used specifically to assess non-hippocampal-driven cognitive function (Kaufman & Lichtenberger, 1999). This task therefore acted as a comparison condition comparing the association of physiological responsivity with hippocampally and non hippocampally-driven declarative memory performance. During this non-timed task participants were shown three example pictures in turn, and were told that each had a missing part to it. They were then asked to look at all aspects of the picture carefully and to choose the missing part from the five options presented. Following this, participants were shown, one at a time, a further set of cards each with a section missing, no feedback was given and the examinee was instructed to make an educated (not random) guess if they were unsure of the correct answer. The procedure ended once the participant had given three consecutive incorrect answers (or until five minutes had passed) (see Appendix VII for instruction sheet). One point was scored for every card correctly identified; scores ranged from 1-26 (with 26 indicating greater cognitive ability). Scaled scores were calculated using the norms detailed in the manual and used in the analyses.

## **2.2.4 Measures**

### **2.2.4.1 Background Measures**

Information concerning chronic illness and medication was obtained from clinical records. The sample in this study suffered from a variety of chronic illnesses such as diabetes, bronchial asthma and arthritis. The presence of chronic illness was taken into account by summing the number of serious medical conditions each

participant reported. Participants were also prescribed a number of medications, so a medication count was computed by summing the number of classes of long-term medication prescribed. These included antihypertensives, statins, steroids, and anti-inflammatory medications (Appendix VIII). Similar measures have been used in a number of other studies of older age (e.g. Benyamini et al, 2004; Seeman et al, 2004). Age of leaving school, number of cigarettes smoked per day, hours of exercise undertaken within a 4 week period and units of alcohol consumed on a typical day were recorded using questionnaires developed for the Whitehall II epidemiological study (Marmot et al, 1991) (Appendix IX). Specific details of hypertensive medication and steroid medication were recorded for use in analyses of blood pressure and cortisol responses. Body weight was measured to the nearest 0.1 kg with participants in underwear, and height was measured to the nearest 0.1 cm. Waist circumference was measured horizontally midway between the lowest rib and iliac crest. Hip circumference was measured as the widest part in the gluteal region. Waist-hip ratio (WHR) was calculated by dividing waist by hip circumference. Body mass index was calculated as weight in kilograms divided by height in metres-squared.

#### **2.2.4.2 Task and Mood ratings**

Each cognitive task was rated for level of difficulty, anxiety, stress, control, involvement and instruction clarity using a seven point Likert scale (Task Appraisal Questionnaire 1-3) (Appendix X). A similar 7-point scale measuring subjective stress, relaxation and anxiety levels was also administered twice during the pre-task and twice during the post task period (Rest Questionnaire A-D) (Appendix XI). Scores on both the Rest and Task Appraisal Questionnaires ranged from 1 = *no stress* to 7 = *very high stress* (for example) on each item.

### 2.2.4.3 Cardiovascular Measures

Blood pressure was assessed using an electronic sphygmomanometer (A&D UA779, Tokyo, Japan). Heart rate, heart rate variability and pre-ejection period were assessed by impedance cardiography using a VU-AMS (Amsterdam, the Netherlands), as described by Willemsen et al (1996). These cardiovascular factors were measured continuously for five minute intervals throughout the test procedure. They were then averaged to produce a mean score for heart rate, heart rate variability and pre-ejection period before during and after the cognitive tasks.

The VU-AMS uses six disposable pre-gelled Ag/AgCl electrodes (Reddot™ 2239-50, 3M Health Care, Germany) to record both electronic and impedance cardiogram signals (ECG/ICG). See Figure 2.1 for fitting instruction diagram. Electrode resistance was kept below 10 k $\Omega$  by first cleaning the skin with alcohol. One electrode, a combined ECG/ICG electrode, was placed 4 cm above the jugular notch on the sternum. The first ‘measuring electrode’ was placed at the apex of the heart over the ninth rib while a ‘ground electrode’ was placed above the right iliac crest. The second ICG ‘measuring electrode’ was placed directly over the tip of the xiphoid process of the sternum. Input impedance of the measuring system was 10 k $\Omega$ . The two current electrodes were placed on the back at the base of the neck (C3/C4) and over vertebrae T8-T9 providing a 50 K-Hz, 350 mA current across the thorax. In brief, a low grade electrical current is then emitted between the current electrodes; the returned current is then receipted by the measuring electrodes. In the VU-AMS, the ECG signal is relayed into a differential amplifier with 1 M $\Omega$  impedance and through a bandpass filter of 17 Hz (Q=33). The R wave peak is recognised with a level detector with automatic level adjustment and at each R wave peak a millisecond counter is read and reset to obtain inter-beat interval (IBI), which are stored continuously. From this ECG recording

measures of heart rate (beats per minute) and heart rate variability were recorded. The rest of the ECG signal is discarded. Heart rate variability was assessed using a time domain measurement. During a 5 minute period the difference between each inter-beat interval was squared, the mean of these squared intervals was then derived and the square root of this mean was calculated as the root mean square successive difference (rMSSD). This approach is advantageous when recording intervals of 5 minutes or more and when stationarity cannot be assumed (Frenneaux, 2004). Heart rate variability measured in this way has been associated with prognosis of post-myocardial infarction, coronary heart disease aetiology, ventricular tachycardia and all-cause mortality (Hemingway et al, 2001).

The VU-AMS also uses the impedance cardiogram signal for measurement of pre-ejection period during 5 minute intervals. The impedance signal is amplified, relayed to a precision rectifier, and filtered at 750 Hz to obtain  $Z_0$ . From  $Z_0$ ,  $dZ$  is obtained by continuously subtracting the integrated  $Z_0$  over the last 10 seconds. The  $dZ$  is differentiated at 33.3 Hz to derive a  $dZ/dt$  that is subsequently passed through a 30 Hz cut-off filter (12 dB/octave roll off). The resulting  $Z_0$ ,  $dZ$ , and  $dZ/dt$  are transmitted to the analogue-to-digital converter of the microprocessor.  $Z_0$  is sampled with a frequency of 10 Hz and  $dZ/dt$  is sampled at 250 Hz. The  $dZ/dt$  values are sampled only during a short period (512 ms) around each R wave and ensemble averaged over 60 seconds.

The VU-AMS is comparable with other spot electrode devices (such as the Nihon Kohden device; Willemsen et al, 1996), and is thought to be more comfortable, unobtrusive and sensitive than traditional band electrode devices. The VU-AMS is now recognised as a reliable and valid measure of cardiac function (Boomsma et al, 1989; Sherwood et al, 1990).

#### **2.2.4.4 Neuroendocrine Measures**

Glucocorticoid activity can be reliably assessed by measurement of free salivary cortisol (Kirschbaum & Hellhammer, 1994). The easiest and most hygienic way to collect saliva is with a Salivette device (Sarstedt, Inc. Leicester, UK). This method is largely stress free and independent of medically trained personnel. The Salivette consists of a sterilised cotton swab, a small beaker and plastic tube. Since flow rate does not influence cortisol levels (as it enters by means independent of active transport; Kirschbaum & Hellhammer, 1994) saliva samples are quickly and reliably obtained by the participant gently chewing on the cotton swab for 120 seconds. Samples obtained during the study were stored at -30 °c. After defrosting, samples were centrifuged at 3000 rpm for five minutes and 100 µl of supernatant was used for duplicate analysis involving a time-resolved immunoassay with fluorescence detection (Dressendörfer et al, 1992). All assays were carried out by Clemens Kirschbaum at the Institute of Experimental Psychology, Dusseldorf. Typically salivary cortisol will peak 10-20 minutes following an event (Clow et al, 1997) so cortisol was measured at regular intervals throughout the procedure (Kirschbaum & Hellhammer, 1994).

#### **2.2.5 Procedure**

A diagrammatic overview of the procedure is detailed in Figure 2.2. Participants were tested individually in a clinic room in the health centre at either 09:00 h or 13:30 h (see Appendix XII for study protocol). A brief structured interview examining current state of health and the previous day's events was then administered (including questions ascertaining sleep quality, level of exercise and amount of alcohol consumed). A series of standard anthropometric measurements was obtained to assess current health status. These included resting blood pressure, heart rate, height, weight, body mass index and

waist to hip ratio. The first saliva sample (sample 1) was then taken for practice reasons, and was not included in the analyses. Following administration of a brief Likert-type questionnaire asking how relaxed, anxious or stressed they felt (rest questionnaire A), subjects were fitted with the VU-AMS and asked to relax (sitting with closed eyes in a darkened room).

The impedance cardiogram monitored cardiovascular activity for 5 minutes (baseline), followed by two readings of blood pressure, two saliva samples (samples 2 & 3) and the baseline rest questionnaire (rest questionnaire B). Participants then performed the first cognitive task (VPA 1). Impedance cardiography continued throughout, and blood pressure, saliva (sample 4), subjective stress (rest questionnaire C) and task appraisals (task appraisal questionnaire 1; TAQ 1) were measured immediately afterwards. Participants then performed the second cognitive task (MR), again followed by task impact ratings (TAQ 2), saliva (sample 5) and blood pressure assessments. The third cognitive test (VPA 2) was then administered before the third impact questionnaire (TAQ 3) and saliva measures (sample 6) were taken. Finally, the participant lay down for 5 min, with impedance measures continuing throughout, and measures of blood pressure, saliva (samples 7 and 8), and stress (rest questionnaire D) were repeated.

### **2.2.6 Data Reduction and Statistical Analyses**

Comparisons of men and women's background characteristics were made using univariate analyses of covariance (ANCOVA), adjusting for age, medication count, chronic illness, and school leaving age.  $\chi^2$  calculations were used for comparison of marital status by gender. Task appraisals were compared across the three tasks using repeated measures analysis of variance (ANOVA) with gender as the between-subject factor.

Cardiovascular data (heart rate, heart rate variability and pre-ejection period) were averaged over six 5 minute trial periods (baseline, task 1, task 2, task 3, immediately post-task, and 10 minutes post-task). Cardiovascular reactivity (HR, HRV and PEP) was determined by subtracting baseline values from measurements taken during each of the three different tasks (higher reactivity indicated greater responsivity to challenge). Task recovery was determined by subtracting recovery values from the measurement obtained during each task (greater recovery value indicated better recovery from the task). During development of the detailed experimental protocol (see Appendix XII) an error meant that blood pressure was not read after task 3 (VPA 2). This design error was only detected after data collection. Since there were rather few blood pressure readings, it was decided not to analyse responses to each task separately. Systolic and diastolic blood pressure (BP) was therefore reduced to three points: baseline, task maximum, and recovery (taken 10 minutes post-task), in order to gain a more robust effect. Blood pressure reactivity was subsequently calculated by subtracting the baseline level from the task level; while blood pressure recovery was calculated by subtracting task levels from baseline levels assessed 10 minutes post-task.

Cortisol was not analysed from participants who were taking steroid medications, so results were based on 107 participants. Preliminary analyses using repeated measures analysis of variance, with steroid use and gender as the between subjects factors, showed no interaction or main effect of steroid use on diastolic or systolic blood pressure. A main effect of steroids was present for heart rate ( $F_{1,96} = 8.22, p = .005$ ), since steroid users had higher heart rates than nonusers. Steroid use was added as a covariate for analyses of heart rate. Heart rate and blood pressure analyses were based on 133 participants.



Cortisol responses to tasks were delayed in comparison with cardiovascular reactions. There were 8 samples of cortisol in total. Sample 1 was discarded, and the baseline for the session was defined as the lower of samples 2 and 3. Inspection of the data indicated that people varied greatly in the time course of cortisol responses to tasks. 32 participants (30 %) showed a peak in cortisol with sample 4, 17 (16 %) with sample 5, 19 (18 %) with sample 6, 24 (22 %) with sample 7, and 15 (14 %) with sample 8. It was not possible to analyse both cortisol reactivity and recovery. Therefore cortisol responses were analysed by selecting the highest post-task value from samples 4–8, with responsivity defined as the change between this value and the baseline level.

Analysis of covariance was used to assess baseline physiological differences between men and women (covariates included age, chronic illnesses, education and medication). Task performance in men and women was also compared using analysis of covariance. Physiological profiles over the session were assessed using repeated measures analysis of variance, with gender as the between-subject factor, and trial (six for subjective stress ratings, heart rate, pre-ejection period and heart rate variability, three for systolic and diastolic blood pressure, and two for cortisol) as the within-subject factor. *Post hoc* comparisons between individual values were made using Tukey's LSD test.

Associations between physiological function and cognitive performance were performed using multiple linear regressions. Before these regressions were carried out, tests of multi-collinearity were conducted to ensure that independent variables were not too closely associated. None of the correlations exceeded the recommended limit of 0.80 which is considered problematic (Katz, 1999). Separate multiple linear regressions were carried out for diastolic/systolic blood pressure, heart rate, heart rate variability, pre-ejection period and cortisol on the three cognitive performance variables (VPA 1,

VPA 2 and Matrix Reasoning). Both reactivity to, and recovery from, the cognitive tasks were analysed.

Stepwise regression models were tested in these analyses. On the first step, control factors including age, gender, medication, chronic illness and age of leaving school were entered into the model. On the second step of each analysis, the physiological factor (e.g. heart rate reactivity) and the interaction between this factor and gender were entered competitively ( $p$  to enter  $<.05$ ). Cortisol responsivity did not vary systematically with time of day of the test session. Nevertheless, time of day was included in cortisol analyses. The analyses of blood pressure also included antihypertensive medication and the presence of hypertension as additional control variables.

In order to determine whether task appraisals mediated associations between physiological responsivity and cognitive performance, the ratings of subjective stress, perceived task difficulty, performance, involvement and controllability were entered into the regression models; it was assessed whether the physiological response effects remained significant when these appraisals were taken into account.

The results of the regression analyses are presented with unstandardised B coefficients and 95 % confidence intervals. Significant effects are illustrated by dividing the physiological reactivity or recovery factors related to cognitive performance into tertiles. Performance scores associated with each tertile and adjusted for covariates were plotted.

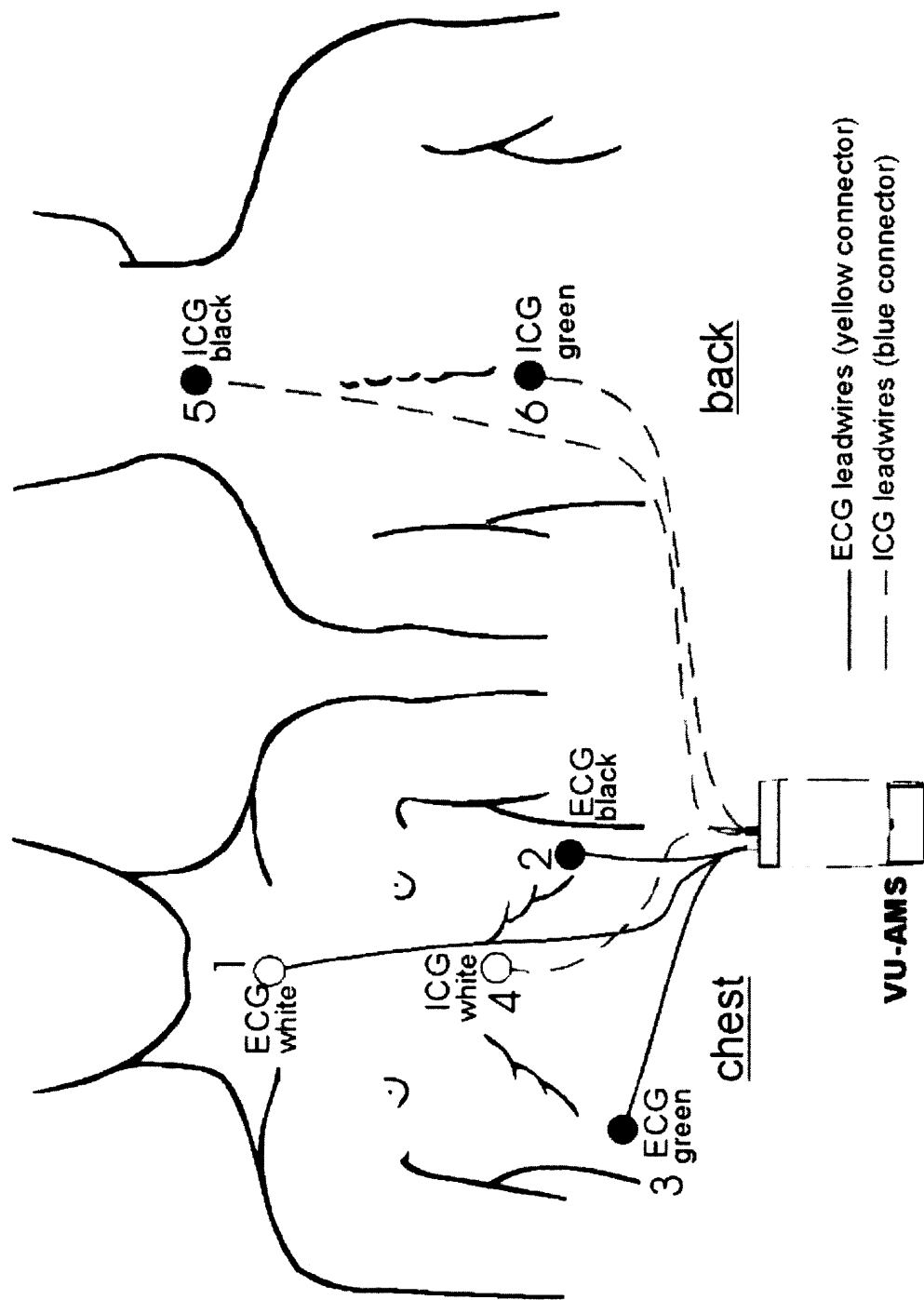


Figure 2.1: Attachment Instructions for VU-AMS Device (Willemssen et al, 1996)

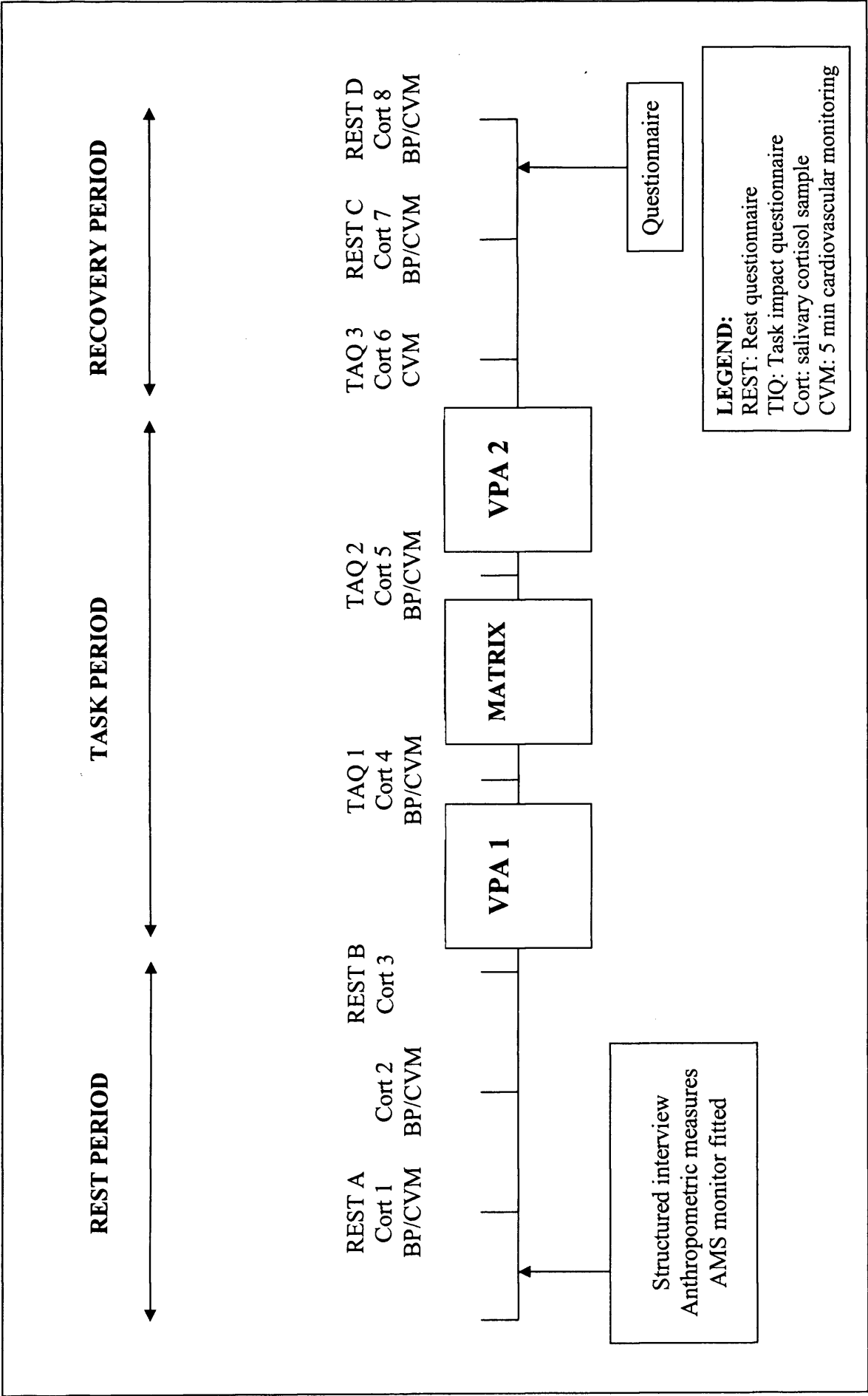


Figure 2.2: Study 1 Procedure Outline

## 2.3 Results

Tables and Figures to accompany this section can be found from page 105.

### 2.3.1 Sample Characteristics

The characteristics of the sample are summarised in Table 2.1. Men were on average 1.4 years younger than women ( $F_{1,126} = 4.75, p = .031$ ), were more likely to be married ( $\chi^2 = 11.6, p = .001$ ), were on average 11.9 cm taller ( $F_{1,126} = 71.0, p = .001$ ), 11.7 kg heavier ( $F_{1,126} = 19.1, p = .001$ ), drank more units of alcohol per day ( $F_{1,106} = 20.5, p = .001$ ) and had a significantly larger waist to hip ratio ( $F_{1,126} = 31.86, p = .001$ ). Conversely, men and women had similar body mass indices ( $F_{1,126} = .14, p = .71$ ), smoked a similar number of cigarettes per day ( $F_{1,77} = .52, p = .46$ ), partook in similar amounts of exercise ( $F_{1,108} = .87, p = .35$ ) and left school at a similar age ( $F_{1,127} = .69, p = .41$ ). The participants had completed education when aged 15.7 years on average, indicating that educational level was generally low. Only 10 individuals (7.5 %) were current smokers. The mean body mass index was  $27.3 (\pm 4.5)$ , indicating that participants were overweight on average. The mean number of medications taken by participants was 1.3 each, these were similar for men and women ( $F_{1,127} = 3.26, p = .07$ ). Thirteen percent of participants took steroid medication, while 54 % took medication for hypertension, these included beta blockers, diuretics, ACE inhibitors and calcium channel blockers. There was no difference between male and female steroid ( $F_{1,122} = 1.69, p = .20$ ) or antihypertensive medication use ( $F_{1,122} = 1.64, p = .20$ ). The sample suffered from an average of 1.3 chronic illnesses each, with 62 % of participants reporting hypertension in particular. The number of chronic illnesses ( $F_{1,127} = .94, p = .33$ ) and evidence of hypertension did not differ by gender ( $F_{1,122} = 2.10, p = .15$ ).

These gender comparisons are consistent with characteristics of a general population and in particular, what one would expect when studying an elderly sample.

### 2.3.2 Baseline Physiological Measures

Baseline cortisol levels averaged 6.18 nmol/l ( $\pm 4.10$ ) and did not differ by gender ( $F_{1,100}=1.12$ ,  $p = .29$ ) when controlling for age, education, time of day, medication, illness and steroid use. Similarly, baseline heart rate, (69.8 bpm  $\pm 10.5$ ), heart rate variability (34.5,  $\pm 29.7$ ) and pre-ejection period (110.9,  $\pm 29.6$ ) values did not differ according to gender (HR,  $F_{1,99} = .15$ ,  $p = .70$ ; HRV,  $F_{1,85} = 1.83$ ,  $p = .18$ ; PEP,  $F_{1,97} = .87$ ,  $p = .35$ ) when controlling for these variables. Diastolic blood pressure, which averaged 77.7 mmHg ( $\pm 9.6$ ) at baseline did not differ by gender ( $F_{1,120} = .042$ ,  $p = .84$ ), but men's systolic blood pressure was on average 8.1 mmHg higher than women's ( $F_{1,124} = 5.97$ ,  $p = .016$ ) after controlling for age, education, medication, number of chronic illnesses, and the presence of hypertension and hypertension medication.

### 2.3.3 Cognitive Performance and Subjective Task Appraisals

The number of correctly remembered word-pairs on VPA 1 and VPA 2 averaged 8.98 ( $\pm 2.84$ ) and 9.61 ( $\pm 2.95$ ) out of a possible 32, while matrix reasoning (MR) performance averaged 11.3 ( $\pm 3.4$ ) of a possible 26. Individuals differed widely in their responses, from 4 to 17 on VPA 1, 5 to 15 on VPA 2 and 5 to 19 on the MR task.

For each of the three cognitive tasks some deviation from normal distribution was detected (VPA 1  $F = .13$ ,  $p = .001$ ; VPA 2  $F = .13$ ,  $p = .001$ ; Matrix  $F = .12$ ,  $p = .001$ ), however, due to the large sample size ( $>100$ ) it is accepted that assumptions of normal distribution were met (Katz, 1999). Pearson's  $r$  correlations show that scores on all three cognitive tests were strongly associated with each other (VPA 1 and VPA 2,  $r =$

.87,  $p = .001$ ; VPA 1 and Matrix  $r = .26$ ,  $p = .002$ ; VPA 2 and Matrix  $r = .31$ ,  $p = .001$ ). Performance of VPA 1, VPA 2 and matrix reasoning tasks correlated with school leaving age ( $r = .16$ ,  $p = .064$ ;  $r = .18$ ,  $p = .045$ ;  $r = .34$ ,  $p = .001$ , respectively). That is, participants who completed their education at a later age performed better on the cognitive tasks. This was expected, since later school leaving age reflects in part intellectual ability. No significant correlations were found between any of the other control variables and memory scores. There was no gender difference on task performance on either the reasoning or memory tests when adjusting for age, medication, chronic illness and school leaving age (VPA 1  $F_{1,125} = 1.76$ ,  $p = .19$ ; VPA 2  $F_{1,125} = 1.34$ ,  $p = .24$ ; Matrix  $F_{1,125} = 1.12$ ,  $p = .29$ ).

The subjective appraisals of the three tasks are summarised in Table 2.2. There were significant differences between tasks on all four ratings ( $F_{2,260} = 13.2$  to  $153.6$ , all  $p < .001$ ). *Post hoc* tests revealed that all differences between tasks were significant, except for the involvement ratings for the MR and VPA 2 tasks. It is evident that participants rated their task performance as progressively better and task difficulty as progressively less over the session, suggesting an order effect. However, task involvement was least for the first task (VPA 1), while the matrix reasoning task was considered to be the least controllable. Men and women did not differ in any task appraisals.

## 2.3.4 Subjective and Physiological Response to Task

### 2.3.4.1 Subjective Stress Ratings

Analyses of variance revealed that participant's subjective stress ratings changed significantly over the 6 experimental time points ( $F_{3,4,405} = 48.5$ ,  $p = .001$ ). *Post hoc* tests indicated that subjective stress levels rose sharply on administration of the first

task ( $p = .001$ ), before returning below baseline on recovery ( $p = .001$ ). Responses for men and women were similar at all time points ( $F_{3,4,405} = .29$ ,  $p = .867$ ) (See Table 2.3).

#### **2.3.4.2 Cortisol Response to Task**

Repeated measures analyses of variance showed a significant increase in cortisol production from baseline to post-task maximum levels ( $F_{1,105} = 89.5$ ,  $p = .001$ ). A time-gender interaction ( $F_{1,105} = 5.5$ ,  $p = .021$ ) and main effect for gender was present ( $F_{1,105} = 6.2$ ,  $p = .014$ ), indicating that men had greater cortisol response to the cognitive tasks than women (Table 2.3).

#### **2.3.4.3 Heart Rate Response to Task**

Heart rate also differed over the trial ( $F_{3,75,379} = 111.2$ ,  $p = .001$ ). However, *post hoc* tests revealed that the cognitive tasks did not induce an increase in heart rate ( $t = -1.12$ ,  $p = .27$ ). Instead, there was a steady but significant decline in heart rate from task 1 to the first recovery period ( $p = .001$ ). Heart rate then rose again from 63.6 bpm ( $\pm 8.8$ ) to 66.7 bpm ( $\pm 9.0$ ) during the final recovery period ( $p = .001$ ) (Table 2.3). This result may be explained by heart rate measurement in a supine and then upright position during these recovery phases. A time-gender interaction ( $F_{3,75,379} = 9.4$ ,  $p = .001$ ), but no main effect for gender was found ( $F_{1,101} = .29$ ,  $p = .59$ ). *Post hoc* analyses revealed that women did not experience any change in heart rate from baseline during the first two tasks, but experienced a decline in heart rate during task 3. In contrast, Figure 2.3 shows that men's heart rate increased significantly during the first task ( $p = .001$ ) before decreasing again during tasks 2 and 3 ( $p = .001$ ). Both men and women experienced a sharp decline in heart rate when lying down 5 minutes post-task (men  $p = .001$ ; women  $p = .001$ ) and an increase in heart rate when participants returned to a seated position (men  $p = .001$ ; women  $p = .001$ ).



#### 2.3.4.4 Blood Pressure Response to Task

A main effect for systolic blood pressure change over time was seen ( $F_{1.8, 228.6} = 34.7, p = .001$ ). *Post hoc* tests indicate a task-related increase in systolic BP ( $t = -6.6, p = .001$ ) followed by a further increase during recovery ( $t = -2.8, p = .006$ ). However this may be explained by a short recovery period and change in participant's position. A time-gender interaction ( $F_{1.8, 228.6} = 4.6, p = .013$ ), but no main effect for gender was found ( $F_{1, 121} = 3.14, p = .079$ ). Analyses revealed that on average men had higher systolic blood pressure at baseline ( $t = 2.3, p = .023$ ) and during the cognitive tasks ( $t = 2.46, p = .015$ ) than women (Figure 2.4). However, while both men and women's blood pressure increased in response to the tasks (men  $p = .001$ ; women  $p = .001$ ) it was only women's systolic blood pressure which continued to rise after testing had finished ( $p = .001$ ; men  $p = .80$ ). In comparison, men displayed a 0.65 mmHg reduction in systolic BP during the recovery period.

A main effect of diastolic pressure was also found ( $F_{2, 252.5} = 46.7, p = .001$ ). Table 2.3 shows a 5.3 mmHg increase in diastolic BP in response to the challenge ( $t = -8.9, p = .001$ ) and then a 1 mmHg decrease during the recovery period. There was no time-gender interaction ( $F_{2, 252.5} = .88, p = .416$ ) or main effect of gender present ( $F_{1, 127} = 1.49, p = .225$ ) (Figure 2.5).

#### 2.3.4.5 Heart Rate Variability and Pre-Ejection Period Response to Task

Mean HRV differed over trial ( $F_{4.5, 399.5} = 3.0, p = .014$ ). *Post hoc* tests suggest a sharp rise in HRV following the second challenge ( $p = .009$ ). No time-gender interaction ( $F_{4.5, 399.5} = 1.72, p = .137$ ) or main effect of gender was found. Table 2.3 indicates that no gender-time interaction ( $F_{3, 295.7} = .70, p = .55$ ) or main effect of PEP ( $F_{3, 295.7} = 1.5, p = .21$ ) or gender ( $F_{1, 98} = 1.7, p = .19$ ) was found.

Despite the modest task-induced changes in physiological variables found, it should be emphasised that individual differences in response were substantial. For example, heart rate responses to the first task (VPA 1) ranged from -11.4 to +10.6 bpm, and recovery effects from -8.9 to +12.4 bpm. Diastolic BP responses to tasks ranged from -12.0 to +33.0 mmHg, and cortisol responses from -2.60 to +12.9 nmol/l.

### **2.3.5 Associations between Task Appraisals and Cognitive**

#### **Performance**

Participants rated feelings of difficulty, involvement, perceived performance, stress and instruction clarity of the task immediately following completion of each cognitive test. Multiple regression analyses for VPA 1 revealed that participants who thought the task was less difficult ( $B = -.62$ , C.I.  $-.86$  to  $-.39$ ,  $p = .001$ ) felt more involved ( $B = .37$ , C.I.  $.01$  to  $.64$ ,  $p = .008$ ) and believed that they had performed the task better ( $B = .85$ , C.I.  $.59$  to  $1.10$ ,  $p = .001$ ) demonstrated greater cognitive performance, when adjusting for age, gender, school leaving age, medication use and chronic illness. An older school leaving age was also associated with memory performance when task appraisals were entered into the model ( $B = .29$ , C.I.  $.01$  to  $.57$ ,  $p = .042$ ). There was no relationship present between ratings of instruction clarity, stressfulness of the task and memory performance. When these task appraisal variables were entered into the model competitively, performance ( $B = .58$ , C.I.  $.26$  to  $.89$ ,  $p = .001$ ), difficulty ( $B = -.33$ , C.I.  $-.61$  to  $-.06$ ,  $p = .018$ ) and involvement ( $B = .27$ , C.I.  $.03$  to  $.51$ ,  $p = .027$ ) ratings were independently associated with memory performance, accounting for a total of 34.6 % of the variance (control factors, 4.7 %; performance, 24.8 %; difficulty, 2.3 %; involvement, 2.7 %).

For the second memory task (VPA 2) multiple regression revealed that only the belief of performing well was significantly associated with task performance ( $B = .36$ , C.I. .06 to .66,  $p = .018$ ) when accounting for age, gender, medication use, chronic illness and age of leaving school. This effect persisted when this variable was entered into the model with all other task appraisals ( $B = .36$ , C.I. .06 to .66,  $p = .018$ ), accounting for 4.3 % of a total 8.3% of the variance. No association between memory and ratings of task-related involvement, difficulty, stress and instruction clarity were observed.

Multiple regression demonstrated that ratings of task difficulty ( $B = .37$ , C.I. .02 to .72,  $p = .037$ ) and involvement ( $B = .42$ , C.I. .01 to .82,  $p = .044$ ) were significantly associated with matrix reasoning scores when adjusting for the same covariates. These effects persisted when the variables were entered into the model with all task appraisals (difficulty,  $B = .46$ , C.I. .11 to .81,  $p = .010$ ; involvement,  $B = .51$ , C.I. .11 to .92,  $p = .014$ ) and accounted for 22.1% of the total variance on matrix scores (controls, 15.2 %; difficulty, 3.0 %; involvement, 4.0 %). There was no association between matrix reasoning scores and task ratings of stress, performance and instruction clarity.

From this analysis it will be possible to determine whether task appraisal factors influence the relationship between cardiovascular and neuroendocrine responsivity and cognitive outcome, or whether physiological response to challenge exerts an independent effect on cognitive performance irrespective of perceived ability. For example, it may be possible to determine whether a participant who is more reactive to the task may also find the task more difficult, feel less involved and believe that they have performed poorly. As a result, it could be these negative task appraisals which account for worse memory performance and increased physiological responsivity.

### 2.3.6 Cortisol Responsivity and Cognitive Performance

Associations between cortisol concentrations and cognitive performance were analysed using linear regression. Scores on the first memory task (VPA 1) were not related to baseline cortisol values ( $t = .14$ ,  $p = .89$ ), but there was a significant association between VPA 1 memory scores and cortisol responsivity that was independent of age, gender, chronic illness, medication use, school leaving age and time of day ( $B = -.18$ ,  $p = .037$ ). However, there were no interactions between these factors and gender ( $t = -.075$ ,  $p = .94$ ). The regression analyses of cortisol reactivity on VPA 1 performance are summarised in Table 2.4, model 1. For illustrative purposes cortisol values were split into tertiles (lower, medium, higher cortisol change scores) and the mean number of correctly recalled word pairs for each group is shown in Figure 2.6a. It is evident that participants who reacted strongly to the cognitive challenge had a poorer memory performance. Overall, high cortisol responders recalled 24 % fewer word-pairs than low cortisol responders. Table 2.4, model 2 shows that the relationship between cortisol reactivity and memory was retained when baseline levels were controlled for ( $B = -.19$ ,  $p = .036$ ), and this did not appear to reduce the amount of variance accounted for, which remained at 4.1 %. In a third model, task appraisals were introduced as control variables. The results indicate that the association of cortisol reactivity and memory performance was reduced to non significance. It was also revealed that task difficulty rating ( $B = -.37$ , C.I.  $-.70$  to  $.04$ ,  $p = .027$ ) and performance rating ( $B = -.49$ , C.I.  $-.08$  to  $.89$ ,  $p = .02$ ), were independently associated with memory score and that cortisol reactivity was no longer significantly related to memory performance.

For the second memory task (VPA 2) multiple regression again revealed no association between baseline cortisol values and cognitive performance ( $t = .17$ ,  $p = .86$ ). Table 2.5, model 1 shows that a significant association was discovered between

VPA 2 memory scores and cortisol responsivity after adjusting for age, gender, chronic illness, medication count, school leaving age and time of day ( $B = -.25$ ,  $p = .006$ ).

Again, no interactions were present for gender ( $t = 1.13$ ,  $p = .26$ ). Figure 2.6b shows that the mean number of word pairs recalled for those in the high reactivity group was 29 % lower than for participants who responded least to the challenge. The associations between cortisol reactivity and memory performance persisted when adjusting for baseline levels ( $B = -.25$ ,  $p = .008$ ; Table 2.5, model 2, step 2) and task appraisals ( $B = -.27$ ,  $p = .005$ ; Table 2.5, model 3, step 2). This suggests that cortisol responsivity may be directly associated with memory performance, and that the relationship is not secondary to variations in subjective appraisals.

No association between the matrix reasoning task and baseline cortisol ( $t = -.65$ ,  $p = .95$ ), or cortisol responsivity ( $t = -.065$ ,  $p = .52$ ) was evident when adjusting for co-variables. A higher school leaving age ( $B = .67$ , C.I. .32 to 1.03,  $p = .001$ ), lower medication count ( $B = -.64$ , C.I. -1.26 to -.012,  $p = .045$ ) and participating in the afternoon ( $B = 1.41$ , C.I. .16 to 2.66,  $p = .028$ ) were all independently related to better task performance. This negative finding is important, since it indicates that the associations between cortisol responsivity and VPA 1 and VPA 2 are specific to memory performance, and do not generalise to the reasoning task.

### **2.3.7 Heart Rate Responsivity and Cognitive Performance**

Multiple regression of memory scores for VPA 1 showed no associations with baseline heart rate ( $t = -.001$ ,  $p = .10$ ) or heart rate reactivity to this task ( $t = 1.64$ ,  $p = .10$ ). Table 2.6, model 1, step 2 shows that a significant association for VPA 1 memory scores and heart rate recovery was discovered after adjustment for age, gender, chronic illness, medication use, steroid medication and school leaving age ( $B = .23$ ,  $p = .006$ ). No interactions were found between these variables and gender ( $t = .32$ ,  $p = .75$ ) but

gender did appear to exert an independent effect on memory score when recovery values were added to the model ( $B = 1.60$ ,  $p = .008$ ). Heart rate recovery was also split into tertiles to form three groups (good, medium and poor recovery scores). As shown in Figure 2.7a, memory performance on this task was better among individuals who had a larger recovery from the cognitive challenge (17 % better). In all, heart rate recovery accounted for 7.4 % of the variance in VPA 1 memory scores. This association persisted when magnitude of task reactivity ( $B = .24$ ,  $p = .004$ ; Table 2.6, model 2) and task appraisals ( $B = .20$ ,  $p = .008$ ; Table 2.6, model 3) were included in the model; although the amount of variance accounted for was reduced to 7.9 % and 5.3 %, respectively.

Multiple regression of VPA 2 scores demonstrated that heart rate at baseline ( $t = -.48$ ,  $p = .63$ ) and in reaction to the task ( $t = 1.85$ ,  $p = .068$ ) was not associated with recall scores. Table 2.7a, summarises the relationship between heart rate recovery and VPA 2 memory scores. It is evident that a significant interaction between gender and heart rate recovery in the prediction of cognitive performance was found ( $B = .22$ ,  $p = .002$ ). Recall scores of participants in high, low and medium tertiles for heart rate recovery; illustrated in Figure 2.7b, show that memory performance was worse for participants with poorer heart rate recovery (17 % worse). Separate analyses were carried out on men and women, and it was found that the association between cognitive performance and heart rate recovery was significant in women ( $B = .44$ ,  $p = .009$ ) but not in men ( $t = 1.74$ ,  $p = .091$ ). This significant association is summarised in Table 2.7b, model 1. The relationship between memory performance (VPA 2) and heart rate recovery for women persisted when magnitude of task reactivity ( $B = .44$ ,  $p = .011$ ; Table 2.7b, model 2) and task appraisals ( $B = .49$ ,  $p = .008$ ; Table 2.7b, model 3) were taken into account. This suggests that heart rate recovery was independently associated

with both VPA 1 and VPA 2 memory task scores. Heart rate recovery in female participants accounted for 11.4 % of the variance in memory scores for VPA 2, this figure increased to 12 % when magnitude of task reactivity and 12.6 % when task appraisals were added to the model.

Multiple regression analysis of Matrix Reasoning scores, showed no association between performance and baseline heart rate levels ( $t = -.20$ ,  $p = .84$ ), heart rate reactivity levels ( $t = -.24$ ,  $p = .81$ ), or heart rate recovery levels ( $t = 1.36$ ,  $p = .18$ ). This confirms that the association of heart rate recovery with the VPA tasks was specific to memory, and not a reflection of a general relationship with cognition.

### **2.3.8 Systolic Blood Pressure Responsivity and Cognitive**

#### **Performance**

Due to problems of multi-colinearity between blood pressure and gender, it was not possible to analyse gender by blood pressure interactions. The inter-correlations between gender and gender by systolic BP recovery, and gender by gender by systolic BP reactivity interactions were  $r = .95$  and  $r = .95$ , respectively. Similarly for diastolic BP, inter-correlations were highly significant between gender and the gender by recovery and reactivity interactions ( $r = .95$  and  $.91$ ). Men and women were therefore analysed separately.

Multiple regression of memory scores for VPA 1 showed no associations with baseline systolic blood pressure for either men ( $t = -.14$ ,  $p = .90$ ) or women ( $t = 1.02$ ,  $p = .31$ ) after adjusting for age, chronic illness, medication count, presence of hypertension, hypertension medication and school leaving age. No association was found between VPA 1 scores and systolic BP reactivity to the task for either men ( $t = -.002$ ,  $p = .99$ ) or women ( $t = -.69$ ,  $p = .50$ ). Similarly, no association between systolic

BP recovery and memory scores was found for either men ( $t = .89$ ,  $p = .38$ ) or women ( $t = 1.52$ ,  $p = .13$ ).

Regression analyses of memory scores for the second task (VPA 2) also revealed no effect of baseline systolic BP (men  $t = -.16$ ,  $p = .88$ ; women  $t = -.08$ ,  $p = .94$ ), systolic BP reactivity levels (men  $t = -.39$ ,  $p = .70$ ; women  $t = -.13$ ,  $p = .90$ ) or systolic BP recovery levels (men  $t = .036$ ,  $p = .97$ ; women  $t = 1.37$ ,  $p = .18$ ) for either men or women when controlling for age, chronic illness, medication count, hypertension, hypertension medication use and school leaving age.

Analyses of Matrix Reasoning scores revealed no association between baseline systolic BP levels (men  $t = -1.45$ ,  $p = .15$ ; women  $t = -1.43$ ,  $p = .16$ ), systolic BP reactivity levels (men  $t = .20$ ,  $p = .84$ ; women  $t = .60$ ,  $p = .55$ ), or systolic BP recovery levels (men  $t = -1.30$ ,  $p = .20$ ; women  $t = 1.84$ ,  $p = .07$ ) when adjusting for co-variables.

### **2.3.9 Diastolic Blood Pressure Responsivity and Cognitive**

#### **Performance**

There was no association between task performance on VPA 1 and baseline diastolic blood pressure in either men or women (men  $t = -.30$ ,  $p = .76$ ; women  $t = -.09$ ,  $p = .93$ ). Table 2.8a, model 1 summarises the multiple regression analyses between diastolic BP and VPA 1 memory performance in men. It is evident that the number of correctly recalled word-pairs was positively associated with diastolic BP reactivity ( $B = .11$ ,  $p = .029$ ), when adjusting for co-variables. Table 2.8a, model 2 also shows that there is a significant association between VPA 1 memory performance and diastolic BP recovery in men ( $B = .15$ ,  $p = .009$ ), indicating that poorer recovery of diastolic BP was associated with a lower memory score. These effects were only present for men (women's reactivity  $t = -.90$ ,  $p = .90$ ; women's recovery  $t = -.47$ ,  $p = .64$ ). As shown in



Figure 2.8a, men who reacted less to the first task achieved an 18.6 % higher recall score than men who reacted more severely to the challenge. Similarly, men who had more successful diastolic BP recovery from the first task also demonstrated 19 % greater memory performance than men who were poor recoverers (Figure 2.8b). In combination, these two factors accounted for 21.9 % of the variance in memory scores. However, because there was a strong inter-correlation between diastolic BP reactivity and recovery levels ( $r = .61$ ,  $p = .001$ ) both factors were entered competitively into the regression model by forward selection. A summary of the analysis in Table 2.8a, model 2 shows that only diastolic BP recovery was significantly associated with memory score. This accounted for 12.7 % of the variance in memory scores and persisted even when magnitude of task reactivity ( $B = .17$   $p = .034$ ; Table 2.8b, model 1) and subjective task appraisals ( $B = .11$ ,  $p = .029$ ; Table 2.8b, model 2) were taken into account.

Similar results were obtained for diastolic BP responsivity and the second memory task. Once again, there was no association between task performance and baseline diastolic blood pressure in either men or women (men  $t = -.29$ ,  $p = .77$ ; women  $t = -.212$ ,  $p = .83$ ). Table 2.9a, model 1 shows that the number of correctly recalled word-pairs was positively associated with diastolic BP reactivity ( $B = .13$ ,  $p = .024$ ), when adjusting for covariates. It is evident that there is also a relationship between diastolic BP recovery and VPA 2 scores, as detailed in Table 2.9a, model 2 ( $B = .16$ ,  $p = .012$ ). Again these relationships were present for men but not women (women recovery  $t = -.155$ ,  $p = .87$ ; reactivity  $t = -.01$ ,  $p = .99$ ). The association is illustrated in Figure 2.9a and Figure 2.9b, where it is evident that the number of successfully recalled word pairs was 18 % lower in the highest than lowest reactivity group and 20 % higher in the larger than smaller recovery group. Together these factors accounted for 21.3 % of the

variance in memory scores. Again a strong inter-correlation existed between diastolic blood pressure recovery and reactivity levels ( $r = .61$ ,  $p = .001$ ). Therefore, both factors were entered competitively into the regression model by forward selection. Table 2.9a, model 2 shows that only diastolic BP recovery was associated with memory performance. This independent effect of recovery in men accounted for 11.7 % of the variance in memory scores and persisted when magnitude of task reactivity ( $B = .18$ ,  $p = .044$ ; Table 2.9b, model 1) and subjective task appraisals ( $B = .17$ ,  $p = .018$ ; Table 2.9b, model 2) were taken into account.

A difference in alcohol consumption was also observed between males and females (Table 2.1). When this factor was entered into the model as an additional covariate, diastolic BP recovery ( $B = 0.14$ , C.I. 0.03 to 0.24,  $p = .015$ ) was still positively associated with memory performance in male participants. No associations were found between baseline diastolic BP and memory performance, or between diastolic BP reactivity or recovery and performance of the matrix reasoning task when alcohol consumption was controlled for.

Finally, analyses of Matrix Reasoning scores, revealed no association between baseline diastolic BP (men  $t = -1.40$ ,  $p = .17$ ; women  $t = -1.39$ ,  $p = .17$ ), diastolic BP reactivity levels (men  $t = 1.01$ ,  $p = .32$ ; women  $t = 1.54$ ,  $p = .13$ ), or diastolic BP recovery levels (men  $t = .31$ ,  $p = .76$ ; women  $t = .82$ ,  $p = .42$ ) when adjusting for covariates.

### **2.3.10 Heart Rate Variability Responsivity and Cognitive Performance**

Multiple regression of memory scores for the VPA 1 revealed no significant associations with heart rate variability at baseline ( $t = 1.43$ ,  $p = .16$ ), in reaction to the cognitive challenge ( $t = -.68$ ,  $p = .50$ ), or during recovery from the task ( $t = .67$ ,  $p = .50$ ), when controlling for age, gender, chronic illness, medication count and school

leaving age. Analyses of heart rate variability during the second memory task (VPA 2) demonstrated that baseline ( $t = 1.31, p = .19$ ), reactivity ( $t = -1.15, p = .25$ ), and recovery heart rate variability levels ( $t = -.34, p = .74$ ) were not significantly associated with memory performance. Similar null results were shown for the Matrix Reasoning task; baseline HRV  $t = -.96, p = .34$ ; HRV reactivity  $t = 1.66, p = .10$ ; HRV recovery.

### **2.3.11 Pre-Ejection Period Responsivity and Cognitive Performance**

Multiple regression of VPA 1 and PEP also revealed no significant associations for either baseline PEP levels ( $t = -.72, p = .48$ ), PEP reactivity to the challenge ( $t = 1.49, p = .14$ ) or PEP recovery from the task ( $t = -.29, p = .77$ ). Similarly, no associations emerged when baseline PEP levels ( $t = 1.65, p = .10$ ), PEP reactivity ( $t = -1.45, p = .15$ ) and PEP recovery ( $t = .51, p = .61$ ) were associated with scores from the second memory task (VPA 2). Lastly, no significant associations were found for the matrix reasoning task and pre-ejection period; PEP baseline  $t = 1.41, p = .16$ ; PEP reactivity  $t = -.997, p = .32$ ; PEP recovery  $t = -.30, p = .77$ .

### **2.3.12 Cortisol Responsivity, Heart Rate Recovery and Memory**

#### **Performance**

In order to determine whether neuroendocrine and cardiovascular mechanisms were independently related to cognitive performance, associations between VPA 1, cortisol reactivity and heart rate recovery were analysed using linear regression. In the first step, age, gender, chronic illness, medication count, school leaving age and time of day were entered into the model. These accounted for 4 % of the variance. Cortisol responsivity and heart rate recovery were then entered in a stepwise fashion (as shown in Table 2.10, model 1). Analyses revealed that a significant association was only

present for heart rate recovery ( $B = .25$ ,  $p = .006$ ), this accounted for 8.8 % of the variance in memory scores.

The same analysis was repeated for VPA 2. Step 1 revealed control variables, accounted for 4% of the total variance. Cortisol responsivity and heart rate recovery levels were then entered competitively into the equation. Heart rate recovery was significantly associated with cognitive performance accounting for 8.7 % of the variance ( $B = .32$ , C.I. .09 to .55,  $p = .008$ ). Step 3, illustrated in Table 2.10, model 2, shows that cortisol responsivity was also independently related to memory performance ( $B = -.25$ ,  $p = .013$ ) accounting for 7.0 % of the variance. Thus, unlike the results for VPA 1, both cardiovascular and neuroendocrine function was related to memory performance.

### **2.3.13 Cortisol Responsivity, Diastolic Blood Pressure Recovery and Memory Performance**

Regression analysis was also used to determine whether cortisol and diastolic blood pressure were both independently associated with performance on the first memory task (VPA 1). Based on previous findings, the analysis was carried out on men. The effects are summarised in Table 2.11. In the first step, age, chronic illness, medication count, school leaving age, antihypertensive medication, presence of hypertension and time of day accounted for 7.8 % of the variance. Cortisol responsivity and diastolic blood pressure recovery levels were then entered in a stepwise fashion. Both cardiovascular (diastolic BP) and neuroendocrine (cortisol) mechanisms were independently associated with memory performance. Step 2 showed that men's diastolic BP recovery was significantly related to memory performance ( $B = .13$ , C.I. .02 to .24,  $p = .018$ ), while step 3 (illustrated in Table 2.11, model 1) shows that cortisol

responsivity was also independently associated with memory scores on the first task ( $B = -.14$ ,  $p = .046$ ). Diastolic blood pressure recovery accounted for 10.9 % of the variance, while cortisol responsivity accounted for 7.1 % of the total variance in memory scores.

Multiple regression analysis was also performed for diastolic BP, cortisol and VPA 2 scores. Cortisol responsivity and diastolic BP recovery were entered competitively into the equation after adjusting for covariates. Results summarised in Table 2.11, model 2. Step 3 revealed that both men's diastolic BP recovery ( $B = -.20$ ,  $p = .006$ ) and cortisol responsivity levels ( $B = .16$ ,  $p = .009$ ) were significantly associated with scores on the second memory task, and that 57.7 % of the variance in the scores was accounted for in total (Controls, 11.1 %; Cortisol, 10.8 %; diastolic BP 11.4 %). It is apparent that diastolic BP recovery and cortisol responsivity are associated with performance on the two memory tasks independently of task appraisals.

**Table 2.1: Study Sample Characteristics (means  $\pm$  sd)**

	<b>Total Sample (n = 133)</b>	<b>Men (n = 57)</b>	<b>Women (n = 76)</b>
<i>Demographic Characteristics</i>			
Age	70.6 $\pm$ 4.1	69.8 $\pm$ 3.9	71.2 $\pm$ 4.1
Age of Leaving School	15.7 $\pm$ 1.7	15.5 $\pm$ 1.8	15.8 $\pm$ 1.7
Marital Status: Married	78 (58.6 %)	43 (75.4 %)	35 (46.1 %)
Single/Divorced/Widowed	55 (41.4 %)	14 (24.6 %)	41 (53.9 %)
Chronic Illness (N)	1.3 $\pm$ 1.2	1.3 $\pm$ 1.3	1.3 $\pm$ 1.2
Hypertension	62 (46.6 %)	27 (47.4)	35 (46.1)
Medications (N): Total	1.3 $\pm$ 1.2	1.1 $\pm$ 1.1	1.4 $\pm$ 1.3
Steroids	13 (9.8 %)	4 (7.0 %)	9 (11.8 %)
Antihypertensives	54 (40.6 %)	22 (38.6 %)	32 (42.1 %)
Weight (kg)	75.1 $\pm$ 15.0	81.8 $\pm$ 13.5	70.1 $\pm$ 14.1
Height (cm)	165.4 $\pm$ 9.6	172.2 $\pm$ 7.1	160.3 $\pm$ 7.9
Body Mass Index (kg/m <sup>2</sup> )	27.3 $\pm$ 4.5	27.6 $\pm$ 4.2	27.2 $\pm$ 4.7
Waist to Hip Ratio	.897 $\pm$ .10	.950 $\pm$ .05	.859 $\pm$ .11
Alcohol Consumption (units per day)	6.2 $\pm$ 6.8	8.9 $\pm$ 8.1	3.6 $\pm$ 4.1
Cigarettes Smoked (per day)	.86 $\pm$ 3.0	.42 $\pm$ 1.8	1.2 $\pm$ 3.7
Exercise Level (hours per 4 wk)	16.4 $\pm$ 17.1	16.4 $\pm$ 17.1	12.2 $\pm$ 13.2
<i>Baseline Physiological Measures</i>			
Cortisol (nmol/l) (n =107)	6.18 $\pm$ 4.10	6.89 $\pm$ 4.9	5.62 $\pm$ 3.5
Heart Rate (bpm) (n =105)	69.8 $\pm$ 10.5	70.1 $\pm$ 10.6	69.8 $\pm$ 10.4
Heart Rate Variability (ms) (n =91)	34.5 $\pm$ 29.7	31.2 $\pm$ 23.3	36.6 $\pm$ 33.3
Blood Pressure (mmHg): Systolic	135.7 $\pm$ 19.4	140.3 $\pm$ 17.1	132.8 $\pm$ 19.8
(n =130) Diastolic	77.7 $\pm$ 9.6	78.8 $\pm$ 9.4	76.0 $\pm$ 9.7
Pre-Ejection Period (ms) (n =103)	110.9 $\pm$ 29.6	107.8 $\pm$ 30.0	113.4 $\pm$ 29.2

**Table 2.2: Subjective Task Appraisal Ratings (means  $\pm$  sd)**

	<b>Task 1 (VPA 1)</b>	<b>Task 2 (Matrix)</b>	<b>Task 3 (VPA 2)</b>
Difficulty	5.49 $\pm$ 1.9	3.7 $\pm$ 1.7	2.1 $\pm$ 1.7
Involvement	4.9 $\pm$ 1.7	5.7 $\pm$ 1.4	5.6 $\pm$ 1.7
Performance	2.2 $\pm$ 1.7	4.2 $\pm$ 1.5	5.4 $\pm$ 1.7
Stress	3.2 $\pm$ 1.8	2.4 $\pm$ 1.4	1.8 $\pm$ 1.3
Control	3.0 $\pm$ 1.9	4.8 $\pm$ 1.7	5.4 $\pm$ 1.7
Instruction Clarity	6.4 $\pm$ 1.3	6.6 $\pm$ .79	6.8 $\pm$ .55

All ratings on 7 point scales ranged from 1 = low and 7 = high

Table 2.3: Subjective Stress Ratings, Cardiovascular and Neuroendocrine Responses to Cognitive Challenge (means ± sd)

	Baseline	Task 1 (VPA 1)	Task 2 (Matrix)	Task 3 (VPA 2)	Recovery
Subjective Stress Ratings	1.67 ± 1.0 <sup>a,d</sup>	3.14 ± 1.8 <sup>b</sup>	2.31 ± 1.4 <sup>c</sup>	1.83 ± 1.3 <sup>d</sup>	1.46 ± 0.9 <sup>e</sup>
Heart Rate (bpm) Men	70.1 ± 10.6 <sup>a</sup>	72.0 ± 9.5 <sup>b</sup>	71.1 ± 10.0 <sup>a</sup>	69.1 ± 9.4 <sup>a</sup>	66.8 ± 9.4 <sup>d</sup>
Women	69.8 ± 10.4 <sup>a</sup>	69.2 ± 9.9 <sup>a,b</sup>	68.7 ± 10.0 <sup>b</sup>	67.1 ± 9.2 <sup>c,d</sup>	66.6 ± 8.8 <sup>d</sup>
Heart Rate Variability (ms)	36.7 ± 2.7 <sup>a</sup>	37.3 ± 2.8 <sup>ab</sup>	34.2 ± 2.3 <sup>ac</sup>	35.9 ± 2.4 <sup>a</sup>	43.8 ± 2.9 <sup>d</sup>
Pre-ejection Period (ms)	110.5 ± 2.7 <sup>a</sup>	107.3 ± 2.7 <sup>b</sup>	105.9 ± 2.6 <sup>bc</sup>	106.0 ± 2.4 <sup>a</sup>	109.4 ± 3.4 <sup>a</sup>
			Task Maximum		
Systolic BP (mmHg): Men	140.3 ± 17.1 <sup>a</sup>		147.2 ± 17.8 <sup>b</sup>		146.7 ± 19.7 <sup>b</sup>
Women	132.8 ± 19.8 <sup>a</sup>		139.0 ± 20.3 <sup>b</sup>		144.9 ± 22.8 <sup>c</sup>
Diastolic BP (mmHg)	77.7 ± 9.6 <sup>a</sup>		83.0 ± 9.2 <sup>b</sup>		82.0 ± 8.9 <sup>b</sup>
			Stress Level		
Cortisol (nmol/l) Men	6.89 ± 4.9 <sup>a</sup>		10.72 ± 5.8 <sup>b</sup>		
Women	5.62 ± 3.3 <sup>a</sup>		7.93 ± 4.0 <sup>b</sup>		

Values on each row that have different superscripts are significantly different from each other (lsd test  $p < .05$ )



Table 2.4: Factors Associated with VPA 1 Cortisol Reactivity

	Model 1; Step 2				Model 2; Step 2			
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)
Age of Leaving School	.23	(-.08 to .54)	1.46	.15	.23	(-.08 to .54)	1.46	.15
Age	-.09	(-.22 to .04)	-1.33	.19	-.09	(-.22 to .04)	-1.36	.18
Medication Count	.31	(-.26 to .89)	1.09	.28	.30	(-.26 to .89)	1.04	.30
Chronic Illness	.10	(-.48 to .68)	.35	.73	.12	(-.48 to .68)	.40	.69
Time of Day	.07	(-1.04 to 1.18)	.12	.90	.09	(-1.04 to 1.18)	.16	.87
Gender	.54	(-.59 to 1.67)	.95	.34	.52	(-.59 to 1.67)	.90	.37
Cortisol Reactivity	-.18	(-.35 to -.01)	-2.12	.037*	-.02	(-.35 to -.01)	-.34	.74
Gender Interaction				ns				
Cortisol Baseline					-.19	(-.36 to -.013)	-2.13	.036*

\* Significant at  $p < .05$ ns  $p > .05$

Table 2.5: Factors Associated with VPA 2 and Cortisol Reactivity

	Model 1; Step 2				Model 2; Step 2				Model 3; Step 2			
	Regression Coefficient	95% Confidence Interval	t value	Sig. p<.05	Regression Coefficient	95% Confidence Interval	t value	Sig. p<.05	Regression Coefficient	95% Confidence Interval	t value	Sig. p<.05
Age of Leaving School	.27	(-.05 to .60)	1.67	.10	.24	(-.10 to .57)	1.39	.17	.26	(-.09 to .62)	1.48	.14
Age	-.05	(-.19 to .09)	-.73	.47	-.06	(-.20 to .09)	-.81	.42	-.04	(-.18 to .10)	-.54	.59
Medication Count	-.08	(-.67 to .51)	-.29	.78	-.12	(-.74 to .50)	-.39	.70	-.22	(-.85 to .41)	-.70	.49
Chronic Illness	.23	(-.37 to .83)	.771	.45	.25	(-.37 to .87)	.80	.43	.29	(-.32 to .90)	.95	.34
Gender	.34	(-.84 to 1.52)	.57	.57	-.21	(-1.41 to .98)	-.36	.72	.27	(-.95 to 1.49)	.44	.66
Time of Day	-.20	(-1.36 to .96)	-.34	.74	.41	(-.81 to 1.63)	.67	.51	.05	(-1.19 to 1.29)	.08	.94
Cortisol Reactivity	-.25	(-.43 to -.07)	-2.80	.006**	-.25	(-.44 to -.07)	-2.70	.008**	-.27	(-.46 to -.09)	-2.89	.005**
Gender Interaction	ns											
Cortisol Baseline					-.04	(-.19 to .11)	-.52	.60				
Difficulty Rating									-.09	(-.57 to .40)	-.35	.73
Involvement Rating									.05	(-.37 to .47)	.25	.80
Performance Rating									.30	(-.19 to .79)	1.22	.23
Stress Rating									.0007	(-.66 to .66)	.002	.99
Control Rating									.02	(-.50 to .55)	.08	.94
Instruction Clarity									.20	(-1.01 to -1.41)	.33	.74

Table 2.6: Factors Associated with VPA 1 and Heart Rate Recovery (\*Significant at p&lt;.05; \*\* Significant at p&lt; .01)

	Model 1; Step 2					Model 2; Step 2					Model 3; Step 2				
	Regression Coefficient	95% Confidence Interval	t value	Sig. p<.05		Regression Coefficient	95% Confidence Interval	t value	Sig. p<.05		Regression Coefficient	95% Confidence Interval	t value	Sig. p<.05	
Age of Leaving School	.11	(-.21 to .42)	.66	.51		.12	(-.19 to .43)	.79	.43		.08	(-.21 to .38)	.57	.57	
Age	-.002	(-.16 to .12)	-.30	.76		-.002	(-.16 to .12)	-.34	.73		.03	(-.10 to .17)	.49	.62	
Medication Count	.38	(-.20 to .95)	1.30	.20		.40	(-.17 to .96)	1.40	.17		.28	(-.25 to .81)	1.06	.29	
Chronic Illness	-.16	(-.76 to .45)	-.54	.60		-.16	(-.75 to .43)	-.54	.59		-.12	(-.64 to .43)	-.39	.70	
Gender	1.60	(.42 to 2.78)	2.69	.008**		1.80	(.62 to 2.99)	3.03	.003**		1.37	(-.31 to 2.44)	2.56	.012*	
Steroid Medication	-1.12	(-2.83 to .59)	-1.31	.20		-1.08	(-2.77 to .60)	-1.28	.21		-.56	(-2.12 to 1.01)	-.71	.48	
Heart Rate Recovery	.23	(.07 to .40)	2.84	.006**		.24	(.08 to .41)	2.97	.004**		.20	(.05 to .35)	2.72	.008**	
Gender Interaction				ns											
Heart Rate Maximum						.13	(-.01 to .26)	1.87	.065						
Difficulty Rating											-.30	(-.62 to .02)	-1.84	.069	
Involvement Rating											.12	(-.20 to .41)	.71	.48	
Performance Rating											.52	(-.14 to .90)	2.73	.007**	
Stress Rating											.31	(-.06 to .68)	1.65	.10	
Control Rating											.19	(-.13 to .51)	1.19	.23	
Instruction Clarity											.11	(-.38 to -.59)	.44	.66	

Table 2.7a: Factors Associated with VPA 2 and Heart Rate Recovery

Step 1						Step 2					
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		
Age of leaving school	.21	(-.13 to .55)	1.21	.23		.15	(-.18 to .48)	.90	.37		
Age	-.04	(-.19 to .11)	-.55	.58		.02	(-.12 to .17)	.33	.74		
Medication count	.05	(-.57 to .68)	.18	.56		.06	(-.54 to .66)	.21	.84		
Chronic illness	-.21	(-.87 to .45)	-.64	.53		-.11	(-.03 to .52)	-.35	.73		
Gender	.99	(-.25 to 2.23)	1.58	.12		1.16	(-.12 to 2.34)	1.94	.056		
Steroid Medication	-1.02	(-2.99 to .86)	-1.08	.28		-1.28	(-3.08 to .51)	-1.42	.16		
Gender interaction						.22	(.09 to .37)	3.27	.002**		
Heart rate recovery											ns

\*\* Significant at p< .01  
ns p> .05

**Table 2.7b: Factors Associated with VPA 2 and Women’s Heart Rate Recovery (\*Significant at  $p < .05$ ; \*\* Significant at  $p < .01$ )**

	Model 1; Step 2				Model 2; Step 2				Model 3; Step 2			
	Regression Coefficient	95% Confidence Interval	t value	Sig. $p < .05$	Regression Coefficient	95% Confidence Interval	t value	Sig. $p < .05$	Regression Coefficient	95% Confidence Interval	t value	Sig. $p < .05$
Age of Leaving School	-.14	(-.62 to .35)	-.57	.57	-.14	(-.58 to .39)	-.56	.58	-.12	(-.67 to .44)	-.44	.66
Age	-.13	(-.33 to .07)	-1.30	.20	-.13	(-.27 to .13)	-1.28	.21	-.11	(-.32 to .11)	-.99	.33
Medication Count	-.34	(-1.05 to .37)	-.96	.34	-.34	(-.98 to .48)	-.95	.34	-.32	(-1.08 to .44)	-.85	.40
Chronic Illness	-.51	(-.25 to 1.26)	1.34	.19	.51	(-.49 to 1.04)	1.31	.20	.37	(-.45 to 1.19)	.91	.37
Steroid Medication	-2.47	(-4.70 to -.23)	-2.21	.031*	-2.47	(-4.7 to -.22)	-2.19	.033*	-2.2.	(-4.74 to .35)	-1.74	.089
Heart Rate Recovery	.44	(.12 to .75)	2.73	.009**	.44	(.07 to .73)	2.64	.011*	.49	(.13 to .84)	2.77	.008**
Heart Rate Maximum					-.003	(-.08 to .010)	-.026	.98				
Difficulty Rating									.11	(-.68 to .90)	.28	.78
Involvement Rating									-.21	(-.82 to .40)	-.68	.50
Performance Rating									.38	(-.28 to 1.04)	1.17	.25
Stress Rating									-.39	(-1.40 to .61)	-.79	.44
Control Rating									-.004	(-.95 to .94)	-.01	.99
Instruction Clarity									-.70	(-2.54 to -1.45)	-.76	.45

**Table 2.8a: Factors Associated with VPA 1 and Men's Diastolic Blood Pressure Reactivity and Recovery**

	Model 1; Step 2				Model 2; Step 2			
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)
Age of Leaving School	.30	(-.11 to .70)	1.48	.15	.30	(-.09 to .69)	1.54	.13
Age	.0002	(-.19 to .19)	.002	1.0	.003	(-.18 to .19)	.03	.98
Medication Count	.36	(-.71 to 1.43)	.67	.50	.09	(-.95 to 1.13)	.17	.87
Chronic Illness	-.17	(-1.01 to .74)	-.38	.70	-.12	(-1.00 to .77)	-.27	.79
Antihypertensive Medication	.51	(-2.07 to 3.09)	.40	.69	1.02	(-1.48 to 3.52)	.82	.42
Hypertension	-1.04	(-3.59 to 1.51)	-.82	.42	-1.09	(-3.59 to 1.51)	-.88	.38
Diastolic BP Reactivity	.11	(.01 to .21)	2.25	.029*				ns
Diastolic BP Reactivity					.15	(.04 to .26)	2.71	.009**

\* Significant at  $p < .05$ \*\* Significant at  $p < .01$ ns  $p > .05$

**Table 2.8b: Factors Associated with VPA 1 and Men's Blood Pressure Recovery (Adjusting for Task Magnitude and Task Appraisals)**

Model 1; Step 2						Model 2; Step 2					
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		
Age of Leaving School	.28	(-.14 to .70)	1.33	.19		.31	(-.03 to .64)	1.86	.071		
Age	-.008	(-.20 to .19)	-.09	.93		.08	(-.09 to .24)	.98	.34		
Medication Count	.05	(-1.03 to 1.12)	.09	.93		.20	(-.78 to 1.18)	.41	.68		
Chronic Illness	-.12	(-1.00 to .78)	-.26	.80		.15	(-.67 to .97)	.37	.72		
Antihypertensive Medication	1.02	(-1.50 to 3.55)	.82	.42		1.03	(-1.19 to 3.25)	.94	.12		
Hypertension	-.95	(-3.60 to 1.70)	-.72	.47		-1.40	(-3.61 to .82)	-1.27	.36		
Task Maximum	-.02	(.14 to .10)	-.35	.73							
Diastolic BP Recovery	.17	(.01 to .32)	2.18	.034*		.11	(.01 to .21)	2.27	.029*		
Difficulty Rating						-.46	(-1.02 to .10)	-1.65	.21		
Involvement Rating						.42	(.09 to .76)	2.53	.015*		
Performance Rating						.45	(-.24 to 1.14)	1.32	.20		
Stress Rating						.45	(.01 to .89)	2.07	.045*		
Control Rating						-.05	(-.47 to .36)	-.27	.79		
Instruction Clarity						-.07	(-.70 to .55)	-.23	.82		

\* Significant at  $p < .05$ \*\* Significant at  $p < .01$

**Table 2.9a: Factors Associated with VPA 2 and Men's Diastolic Blood Pressure Reactivity and Recovery**

	Model 1; Step 2					Model 2; Step 2				
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)	
Age of Leaving School	.37	(-.08 to .82)	1.65	.11		.37	(-.09 to .69)	1.67	.10	
Age	.04	(-.17 to .26)	.45	.65		.006	(-.18 to .19)	.533	.60	
Medication Count	.07	(-1.13 to 1.28)	.12	.91		-.24	(-.95 to 1.13)	-.41	.69	
Chronic Illness	-.10	(-1.12 to .92)	-.20	.84		-.03	(-1.00 to .77)	-.05	.96	
Antihypertensive Medication	.54	(-2.07 to 3.09)	.38	.71		1.13	(-1.48 to 3.52)	.80	.43	
Hypertension	-1.14	(-2.35 to 3.44)	-.80	.43		-1.20	(-3.59 to 1.51)	-.86	.40	
Diastolic BP Reactivity	.13	(.02 to .24)	2.34	.024*					ns	
Diastolic BP Recovery						.16	(.04 to .26)	2.62	.012*	

\* Significant at p&lt; .05

ns p&gt; .05



Table 2.9b: Factors Associated with VPA 2 and Men’s Diastolic Blood Pressure Recovery (Adjusting for Task Maximum and Task Appraisals)

Model 1; Step 2						Model 1; Step 2					
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)		
Age of Leaving School	.36	(-.12 to .83)	1.51	.14		.39	(-.08 to .86)	1.68	.10		
Age	.11	(-.71 to .94)	.28	.78		.07	(-.15 to .29)	.61	.54		
Medication Count	-.28	(-1.51 to .95)	-.45	.65		.03	(-1.22 to 1.28)	.04	.97		
Chronic Illness	-.03	(-1.04 to .99)	-.05	.96		-.23	(-1.35 to .75)	-.57	.57		
Antihypertensive Medication	1.14	(-1.73 to 4.00)	-.80	.43		1.28	(-1.62 to 4.18)	.89	.38		
Hypertension	-1.09	(-4.09 to 1.91)	-.73	.47		-.73	(-3.74 to 2.29)	-.49	.63		
Diastolic BP Task Maximum	-.01	(.15 to .12)	-.22	.83							
Diastolic BP Recovery	.18	(.01 to .35)	2.07	.044*		.17	(.03 to .30)	2.47	.018*		
Difficulty Rating						-.17	(-.87 to .54)	-.47	.64		
Involvement Rating						.09	(-.44 to .61)	.33	.74		
Performance Rating						.04	(-.63 to .70)	.12	.91		
Stress Rating						.29	(.53 to 1.12)	.72	.48		
Control Rating						-.05	(-.80 to .70)	-.14	.89		
Instruction Clarity						1.82	(-.08 to 3.71)	1.93	.061		

\* Significant at p< .05

**Table 2.10: Factors Associated with VPA 1 and VPA 2, Heart Rate Recovery and Cortisol Reactivity**

		Model 1; Step 2 (VPA 1)				Model 2; Step 3 (VPA 2)			
		Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)
Age of Leaving School		.08	(-.27 to .43)	.46	.65	.15	(-.23 to .51)	.80	.43
Age		-.01	(-.16 to .14)	-.18	.85	-.05	(-.22 to .10)	-.73	.47
Medication Count		.39	(-.21 to .99)	1.30	1.0	-.11	(-.75 to .53)	-.35	.73
Chronic Illness		.03	(-.67 to .67)	-.01	1.0	.29	(-.44 to 1.02)	.78	.44
Time of Day		.11	(-1.15 to 1.38)	.18	.86	-.17	(-1.46 to 1.13)	-.26	.80
Gender		1.52	(.23 to 2.81)	2.35	.021*	1.01	(-.36 to 2.38)	1.47	.14
Heart Rate Recovery		.25	(.071 to .42)	2.80	.006**	.31	(.08 to .53)	2.70	.009**
Cortisol Reactivity					ns	-.25	(-.44 to -.05)	-2.53	.013*

\* Significant at p&lt; .05

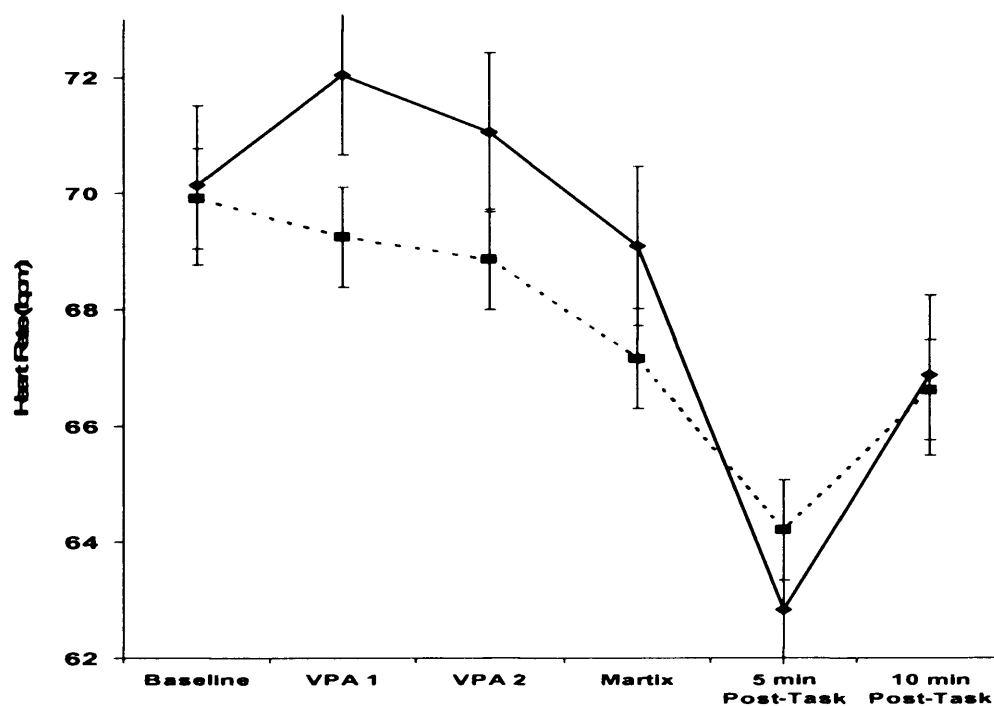
\*\* Significant at p&lt; .01

ns p&gt; .05

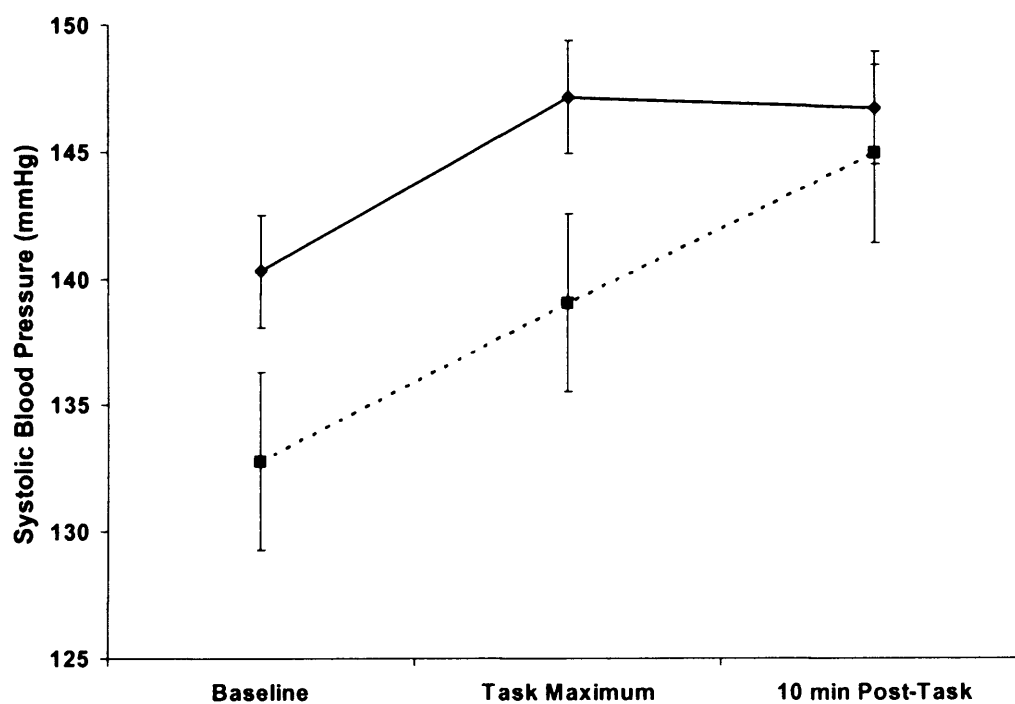
**Table 2.11: Factors Associated with VPA 1 and VPA 2, Men's Diastolic Blood Pressure Recovery and Cortisol Reactivity**

	Model 1; Step 3 (VPA 1)				Model 2; Step 3 (VPA 2)			
	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)	Regression Coefficient	95% Confidence Interval	t value	Sig. (p<.05)
Age of Leaving School	.32	(-.08 to .72)	1.63	.11	.33	(-.10 to .76)	1.54	.13
Age	-.0005	(-.19 to .16)	-.01	.99	.05	(-.15 to .26)	.54	.59
Medication Count	-.10	(-1.12 to .92)	-.20	.84	-.50	(-1.60 to .60)	-.92	.37
Chronic Illness	.15	(-.73 to 1.02)	.34	.74	.35	(-.60 to 1.29)	.74	.47
Time of Day	-.15	(-1.69 to 1.39)	-.20	.84	-.11	(-2.73 to .59)	-1.30	.20
Hypertension	-.74	(-3.16 to 1.68)	-.62	.54	-.67	(-3.28 to 1.94)	-.52	.61
Antihypertensive Medication	.83	(-1.64 to 3.30)	.687	.50	1.04	(-1.63 to 3.70)	.78	.44
DBP Recovery	.14	(.03 to .25)	2.61	.012*	-.20	(-.34 to -.06)	-2.77	.006**
Cortisol Reactivity	-.14	(-.27 to -.003)	-2.06	.046*	.16	(.04 to .27)	2.75	.009**

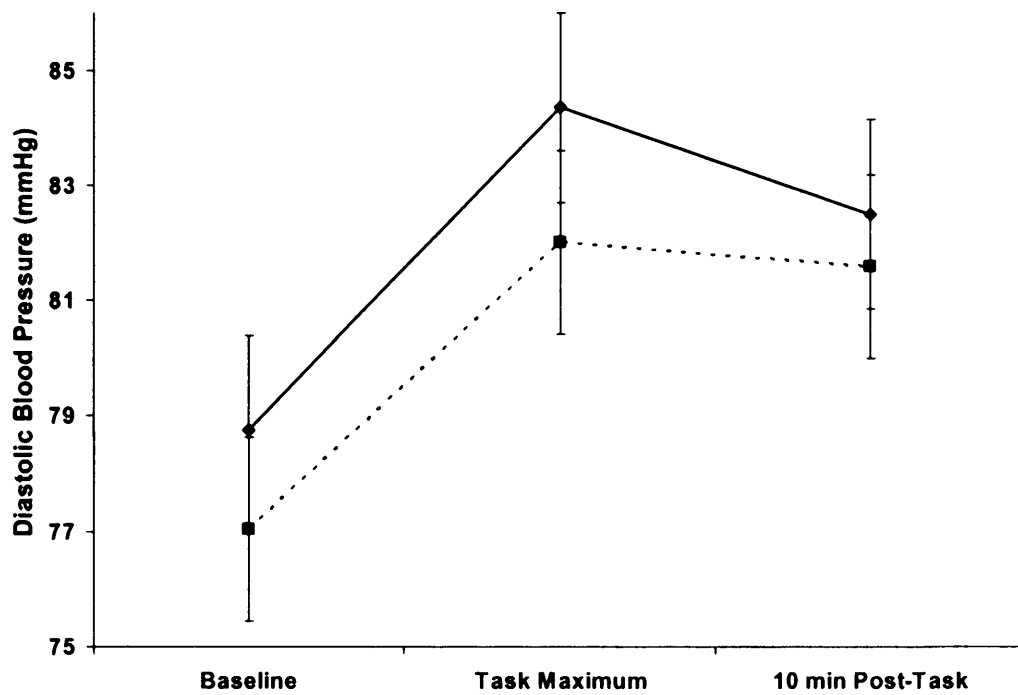
\* Significant at  $p < .05$ \*\* Significant at  $p < .01$ ns  $p > .05$



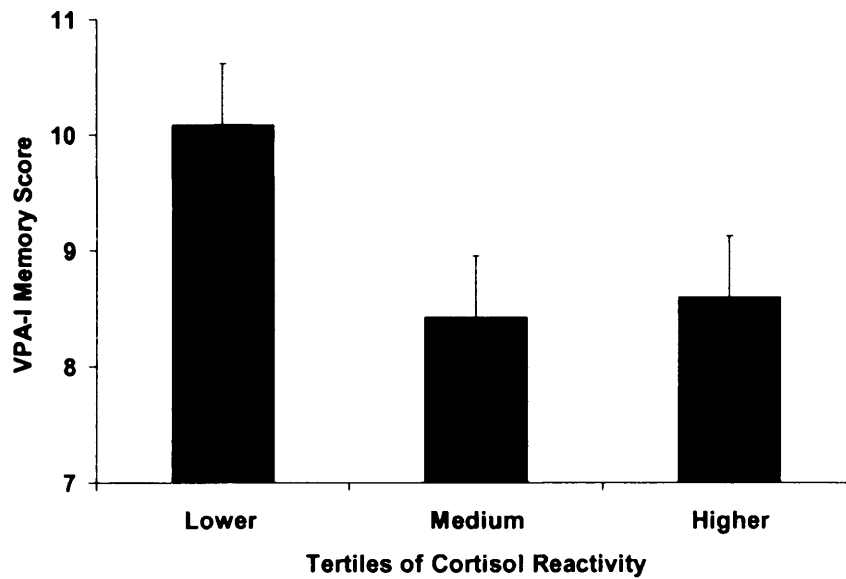
**Figure 2.3: Mean Heart Rate for Men (solid line) and Women (dashed line) over the Study Period. Error bars are standard errors of the mean.**



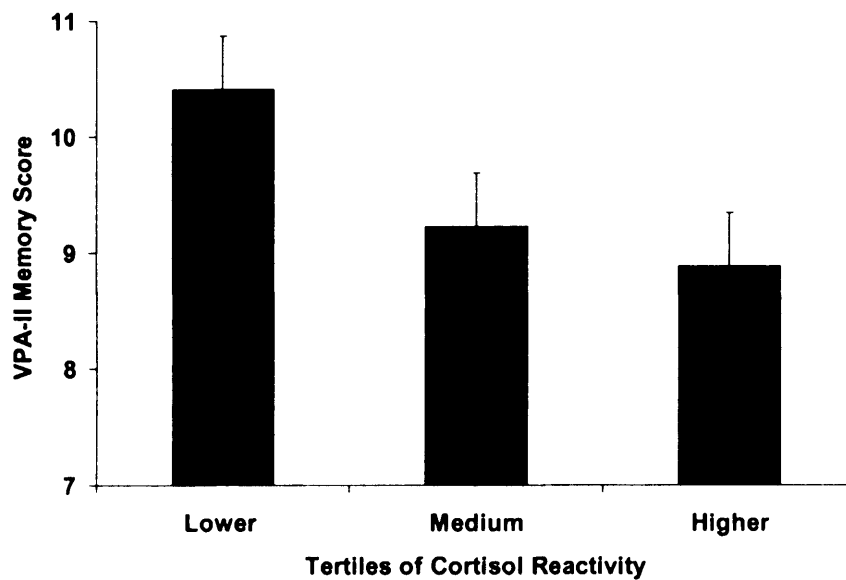
**Figure 2.4: Mean Systolic Blood Pressure for Men (solid line) and Women (dashed line) over the Study Period. Error bars are standard errors of the mean.**



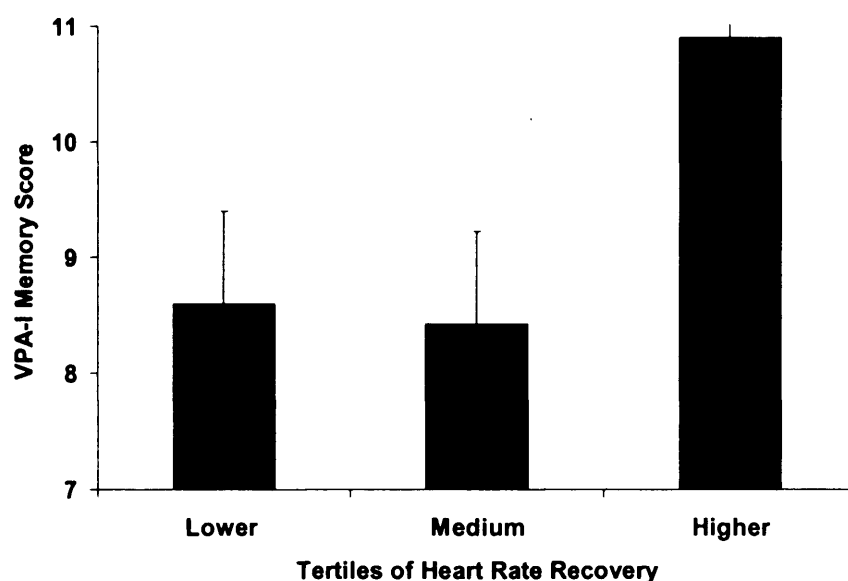
**Figure 2.5: Mean Diastolic Blood Pressure for Men (solid line) and Women (dashed line) over the Study Period. Error bars are standard errors of the mean.**



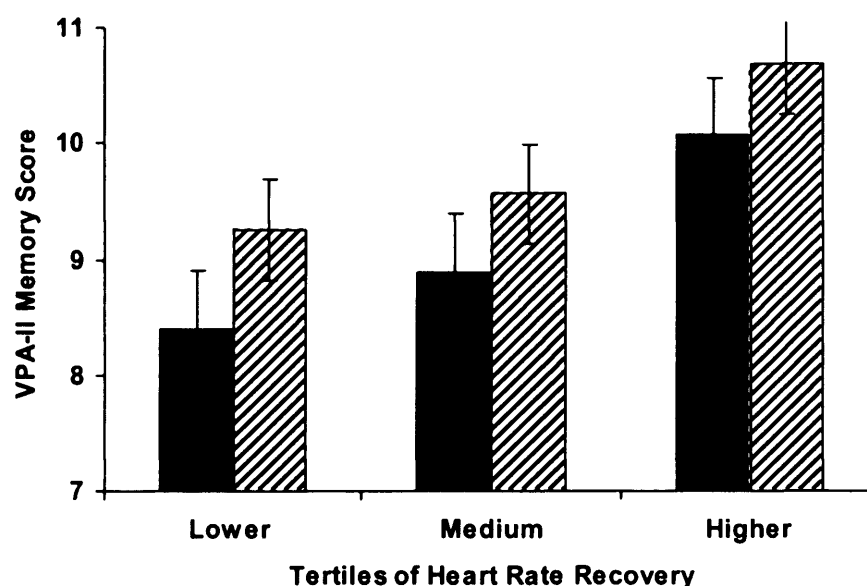
**Figure 2.6a: VPA 1 Scores Achieved by Individuals in Lower, Middle and Higher Cortisol Responsivity Tertiles. Error bars are standard errors of the mean.**



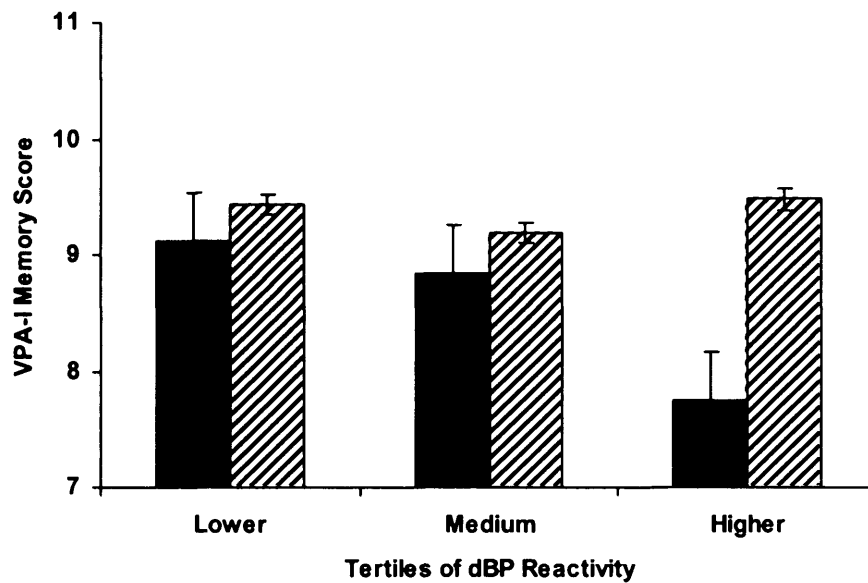
**Figure 2.6b: VPA 2 Scores Achieved by Individuals in Lower, Middle and Higher Cortisol Responsivity Tertiles. Error bars are standard errors of the mean.**



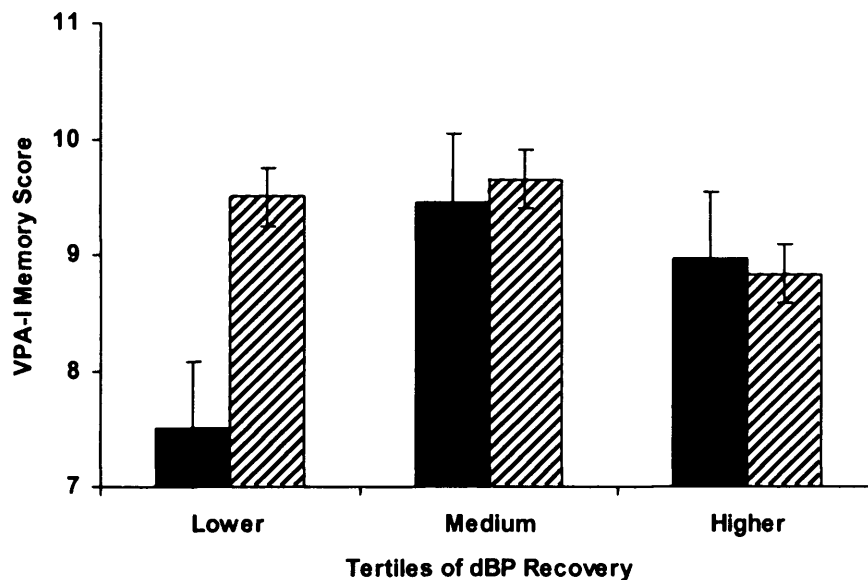
**Figure 2.7a: VPA 1 Scores Achieved by Individuals in Lower, Middle and Higher Heart Rate Recovery Tertiles. Error bars are standard errors of the mean.**



**Figure 2.7b: VPA 2 Scores Achieved by Men (solid bars) and Women (hatched bars) in Lower, Middle and Higher Heart Rate Recovery Tertiles. Error bars are standard errors of the mean.**

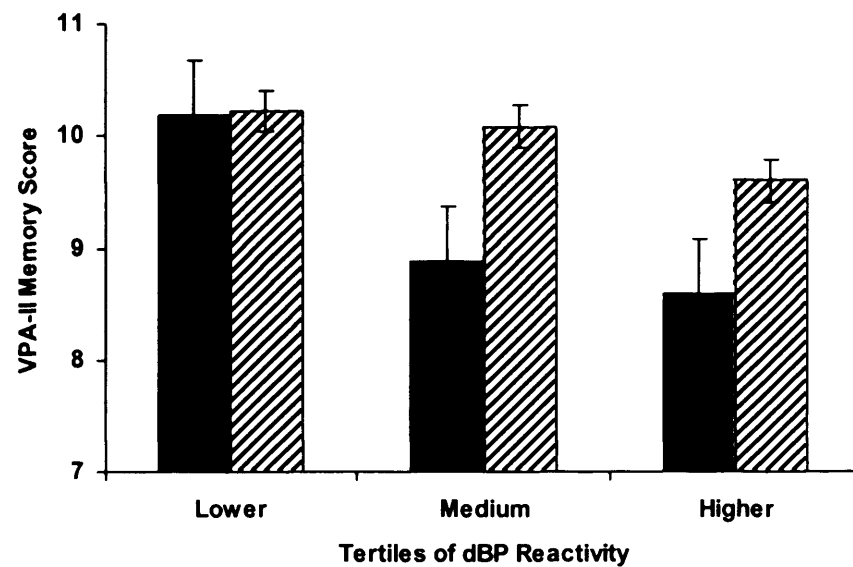


**Figure 2.8a: VPA 1 Scores Achieved by Men (solid bars) and Women (hatched bars) in Lower, Middle and Higher Diastolic Blood Pressure Reactivity Tertiles. Error bars are standard errors of the mean.**

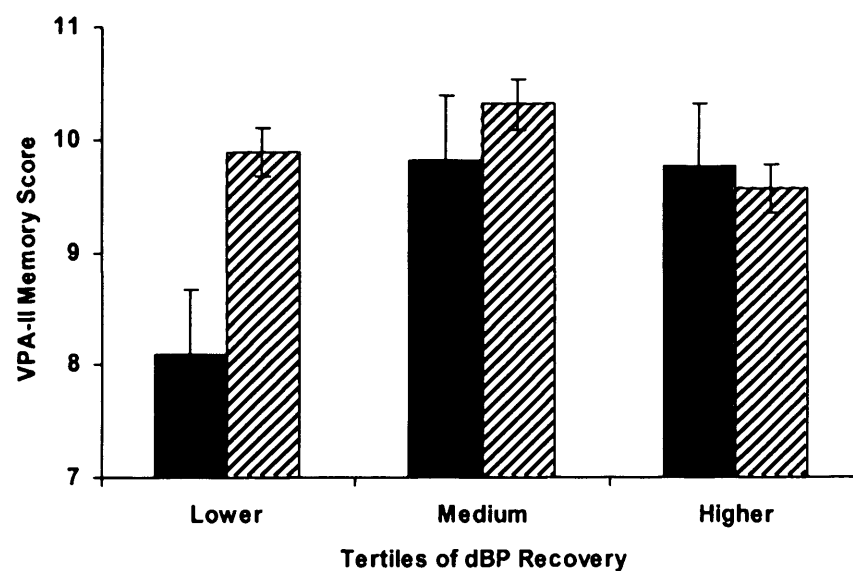


**Figure 2.8b: VPA 1 Scores Achieved by Men (solid bars) and Women (hatched bars) in Lower, Middle and Higher Diastolic Blood Pressure Recovery Tertiles. Error bars are standard error of the mean.**





**Figure 2.9a: VPA 2 Scores Achieved by Men (solid bars) and Women (hatched bars) in Lower, Middle and Higher Diastolic Blood Pressure Reactivity Tertiles. Error bars are standard error of the mean.**



**Figure 2.9b: VPA 2 Scores Achieved by Men (solid bars) and Women (hatched bars) in Lower, Middle and Higher Diastolic Blood Pressure Recovery Tertiles. Error bars are standard error of the mean.**

## **2.4 Discussion**

Cognitive decline varies substantially as people age. Associations have been observed with neuroendocrine responsivity, but the relevance of other physiological processes is unclear. It was hypothesised that greater cortisol and/or cardiovascular responsivity would be associated with worse declarative memory in an elderly sample.

In summary, it appears that the three cognitive challenges (VPA 1, VPA 2, Matrix Reasoning) did produce significant increases in self-rated stress, cardiovascular and neuroendocrine parameters. In addition, it was shown that physiological mechanisms were associated with declarative memory, but not cognitive reasoning performance. The results suggest that the relationship between physiological processes and memory performance is independent of age, medication, chronic illness, age of leaving school, stress of the task or perceived task performance. The associations of cardiovascular measures and memory performance were related to rate of post-task recovery rather than reactivity, while larger neuroendocrine reactions to the task (as indicated by greater cortisol increase) were associated with poorer memory performance. However, the associations between physiological variables and memory are only present for cortisol, diastolic blood pressure and heart rate (and absent for heart rate variability, systolic blood pressure and pre-ejection period). Interestingly, the relationship between cortisol, diastolic blood pressure, heart rate and memory shows that the cardiovascular and neuroendocrine systems appear to be functioning independently of each other, and that these relationships were stronger for the second memory task. The importance of each of these finding is discussed herein.

### **2.4.1 Physiological Responsivity to the Cognitive Tasks**

The cognitive tasks were administered with their standard instructions in a low-stress context without criticism or ego threat. Scores on both memory tasks and the matrix reasoning task compared favourably with norms for this age group. The tasks were not designed as stress tests. However, they still produced significant physiological responses, albeit small, compared with those elicited by traditional stress induction procedures (for example combined cognitive/public speaking tasks such as the Trier Social Stress Test; Dickerson & Kemeny, 2004). There were also wide variations in physiological responsivity between participants. One of the reasons that some studies may fail to show associations between physiological responsivity and memory could be restricted range, and insufficient individual variability which will reduce the likelihood of obtaining robust correlations.

### **2.4.2 Association between Cortisol Responsivity and Memory**

#### **Performance**

Results for this investigation showed no association between memory and baseline cortisol levels. These findings provide support for previous literature. For instance, Wolf et al (2002) reported no association between daily cortisol levels and performance in a paired-associate declarative memory task. Similarly, Wolf et al (2001b) and Seeman et al (1997a) found no relationship between memory and cortisol in an elderly sample. It was proposed that these contradictory findings may be due to the study's failure to account for individual variability in cortisol response, especially when faced with a challenging situation. As a result the present study hypothesised that individual increases in challenge-induced cortisol levels would differ greatly, and that

these differences (in responsivity) would be what relates increased cortisol to poorer memory function (not as some propose, a result of raised basal glucocorticoid levels).

Cortisol levels were assessed at eight regular time intervals throughout the test procedure. To determine reactivity levels, baseline measurement was subtracted from the peak value. In accordance with the proposed argument, results revealed that individuals with high cortisol responsivity to the tasks ('high responders') demonstrated poorer memory performance. High responders recalled 24 % fewer words on their first task and 29 % fewer words on the second memory task than those who responded less severely to the test. The results were found to be independent of age, gender, medication, illness and education. Patients taking steroid medication were omitted from the analyses, so this factor did not contribute to the findings. The association between cortisol reactivity and memory persisted when baseline values were taken into account. In addition, gender did not moderate the cortisol-memory relationship. However there was a significant gender difference in the cortisol response, with men exhibiting a greater response to the challenge. This is consistent with several studies showing that men secrete more cortisol in response to different tasks (Kudielka et al, 1998; Earle et al, 1999).

Although cortisol responsivity was significantly related to cognitive performance, cortisol concentrations in response to the cognitive challenge were relatively low in comparison to previous studies that administered standardised stress tests. In the present investigation mean cortisol levels rose significantly from  $6.18 \pm 4.1$  nmol/l to  $9.16 \pm 5.04$  nmol/l. However, only 44 % of participants showed a clear-cut cortisol response to the tasks marked by an elevation of more than 2.5 nmol/l of cortisol (as recommended by Kirschbaum et al, 1996). This compares to concentrations found in previous studies where cortisol levels rose from 8.46 nmol/l to 17.65 nmol/l

(Kirschbaum et al, 1996) and 10.4 nmol/l to 20.9 nmol/l (Wolf et al, 2001b). Such findings were partly expected as previous studies presented cortisol response to standardised psychological stress tests, whereas the present investigation employed a cognitive challenge (similar to an everyday stressor which might be present in everyday life), which was not primarily designed to induce a stress response. These results therefore add a degree of caution to any interpretation of the findings.

The results appear to support the findings of Kirschbaum et al (1996), who reported that memory performance in young men, was impaired only in participants who had an elevated cortisol response to a task. Similarly, Lupien et al (1997) found that regardless of task-group allocation, elderly participants who were high cortisol responders showed significantly worse memory performance. Domes et al (2002) reported a positive relationship between memory performance and cortisol reactivity, but as previously discussed this study had a number of methodological limitations. The current investigation therefore included certain methodological refinements; various controls were imposed, a relevant challenge was administered and multiple cognitive tasks were employed. Moreover, an attempt was made to investigate the role of motivational and attentional task appraisals on memory performance (as outlined by (Kelly et al, 1996; Hess et al, 2003)). The nature of the relationship between cortisol responsivity and poor memory performance is at present unclear, partly because of the failure to determine whether an increased cortisol response is associated with memory performance independently of task appraisals, or whether task appraisals mediate the relationship between cortisol and cognition.

In this particular study, cortisol responsivity to the first memory task (VPA 1) was negatively related to task performance; however, this effect disappeared when task appraisals were controlled for. Results revealed that participants who believed the tasks

to be difficult, felt less involved, and thought that they had performed poorly, scored worse on the first memory task. These individuals also had higher cortisol responsivity levels. As a result, it could be suggested that appraisals of the task could have produced a greater cortisol response, and a lower score on the first memory task independently of the cortisol response. That is, in certain individuals, task appraisal could have mediated the relationship between cortisol response and cognitive performance. This factor is often neglected in research on older adults.

By the time participants completed the second memory task (VPA 2) they rated the challenge as easier, reportedly felt more involved and thought that they had performed the task well. Only perceived performance was significantly associated with memory score on the second task. During this task cortisol was significantly negatively associated with memory even when the effect of task appraisals had been controlled for. Although the present study found that participants who believed the task to be difficult, felt less involved or thought that they had performed poorly, scored worse on the first memory task, of the six regressions that were performed, only one association (cortisol responsivity and VPA 1) was affected by the inclusion of task appraisals. Because of this it is contended that subjective task appraisals are probably not a ‘third variable’ responsible for both the increase in cortisol and poor memory performance.

This association is further supported by the findings of the Matrix Reasoning task. An association was present between beliefs of better performance, greater involvement, a higher degree of reported difficulty and matrix performance scores. However, no association was found between cortisol reactivity and reasoning performance. One would expect to find this if the relationship between task performance and cortisol was due to the mediating effects of task appraisals. That no

associations were found for the reasoning task suggests that neuroendocrine responsivity may be related to memory and not cognition in general.

Although a significant association was discovered between declarative memory performance and cortisol responsivity to challenge, it was also proposed that poor recovery of cortisol levels would be related to poor memory performance. Seeman and Robbins (1994) state that in addition to the initial reactivity period a recovery phase where cortisol values return to baseline levels exists. This recovery phase is characterised by individual variability, with some participants displaying a poorer or longer recovery period (Seeman & Robbins term these people ‘less resilient’). It was proposed therefore that individuals with poor cortisol recovery would also show poorer memory performance. Unfortunately, due to practical limitations of studying an elderly sample, it was not possible to test participants for long enough to examine an adequate recovery phase of cortisol release.

In summary, results of the present investigation indicate that cortisol responsivity, and not baseline levels, are negatively associated with declarative memory performance independent of age, medication, illness, education, time of day, baseline values and task appraisals, and that this relationship is strengthened as the challenge builds (during VPA 2). In contrast, no associations were found between cortisol levels and the Matrix Reasoning performance, suggesting that cortisol responsivity may be related to declarative memory and not cognition in general.

#### ***2.4.2.1 Mediatory Pathways Associating Cortisol Responsivity and Memory Function***

Declarative memory is believed to be hippocampally driven (Squire, 1992). These results therefore support the notion that the relationship between cortisol and memory could be mediated by the hippocampus. In general, four main arguments have been used to confirm this stress-hippocampus link (Lupien & Lepage, 2001). The first

relates to the presence of glucocorticoid receptors in the hippocampus. The second concerns the association between declarative memory impairment and glucocorticoids, as previously discussed. The third relates to the evidence that chronic exposure to high levels of stress hormones is associated with hippocampal atrophy, and the fourth concerns the effects of stress on hippocampal neurogenesis.

It is thought that dysregulation of the HPA axis (or heightened cortisol production) increases exposure of the hippocampus to elevated glucocorticoid levels. This in turn contributes to declines in declarative memory. The effects of glucocorticoids on the hippocampus depend on their concentration of glucocorticoids. Normal basal levels of cortisol facilitate hippocampal plasticity (Joëls, 1997) and promote neurogenesis of gyrus granule cells (Sloviter et al, 1989), whereas elevated glucocorticoid levels have been associated with hippocampal dysfunction. These apparently contrasting effects can be understood in terms of known differences in corticosteroid receptor subtypes. Mineralocorticoid receptors bind glucocorticoids with a five-to-ten fold higher affinity than glucocorticoid receptors (Reul & De Kloet, 1985). Basal glucocorticoid levels act on the brain via mineralocorticoid receptors, whereas the compromising effects of stress involve activation of the more prevalent glucocorticoid receptors (Sapolsky, 1992). Normal basal cortisol levels primarily activate mineralocorticoid receptors and facilitate hippocampal long-term potentiation (a synaptic model of memory: Joëls, 1997). In contrast, the elevated cortisol levels of the 'high reactivity' individuals, would activate a larger proportion of the glucocorticoid receptors, serving not only to negate the effects of mineralocorticoid receptor activation but also to promote the debilitating glucocorticoid receptor effects on hippocampal function including dampening of declarative memory (Kerr et al, 1989; Diamond et al, 1992). This glucocorticoid-cascade hypothesis subsequently predicts that the



participant's response to this particular challenge is typical of their reaction to stressful situations over the life course and that this cumulative exposure to high levels of glucocorticoids is what compromises hippocampal integrity and impairs memory (Sapolsky et al, 1986).

It has also been shown that chronic exposure to high levels of corticosteroids leads to hippocampal atrophy in both animals (Landfield et al, 1978; Landfield et al, 1981) and humans (Ling et al, 1981; Lupien et al, 1998). For example, Starkman et al (1992) reported a negative correlation between declarative memory and hippocampal formation volume, and between hippocampal formation volume and plasma cortisol levels. Furthermore, Lupien et al (1994; 1998b) demonstrated that elderly participants with prolonged cortisol elevations showed a 14 % reduction in hippocampal volume and declarative memory compared with normal cortisol controls. Finally, stressful experiences can decrease the number of adult-generated neurons in the dentate gyrus of various species, including the rat (Heale et al, 1994; Galea et al, 1996; Sgoifo et al, 1996), tree shrew (Gloud et al, 1997) and marmoset (Gloud et al, 1998). Studies performed in adult neurogenesis also show that learning can enhance the number of granule neurones in the dentate gyrus (Lupien & Lepage, 2001)

Despite not having neuroimaging data for participants of this particular study, it can be tentatively suggested that 'high cortisol responders' with poorer declarative memory performance could have some degree of hippocampal abnormality. Such evidence would have important clinical implications. For example, 80 % of patients with some evidence of cognitive impairment go on to develop a senile dementia of the Alzheimer's type (De Leon et al, 1993). Follow-up investigation of medical records for this sample would be of great interest. Furthermore, recent evidence has suggested that the effects of cortisol on the brain can be reversed, because hippocampal structure

formation (Starkman et al, 1992) and memory function (Starkman et al, 2003), are seen to improve after treatment to lower cortisol production. However, at the moment, evidence of hippocampally mediated memory decline is based solely on the association with raised basal cortisol levels. The real challenge is determining whether individual difference in vulnerability to stress (and cortisol responsivity) produce similar effects on hippocampal structure. In doing so, one would also need to look at the influence of other aspects of the HPA Axis and brain structure in relation to memory. For example, it has been suggested that stress and glucocorticoid-induced activation of noradrenergic mechanisms in the basolateral amygdala could be responsible for co-ordinating memory consolidation (Roozendaal, 2000). Likewise, glucocorticoids could induce a decrease in glucose transport that would impair long-term-potential in the hippocampus (McEwen & Sapolsky, 1995); while it has also been proposed that adrenaline, noradrenaline corticotrophin releasing hormone and adrenocorticotrophic hormone (ACTH) released in response to stress may affect hippocampal atrophy, thus reducing memory performance.

### **2.4.3 Association between Blood Pressure and Memory Performance**

An abundance of evidence exists exploring the relationship between memory performance and blood pressure at basal levels or during a stressful event. However, the literature has produced varied results, with some authors reporting a negative association between blood pressure and memory (Wallace et al, 1985; Cacciato et al, 1997; Budge et al, 2002; Izquierdo-Porrera & Waldstein, 2002; Pandav et al, 2003), and others reporting no association between the two variables (Van Boxtel et al, 1997; Glynn et al, 1999). Results from the present study also discovered no relationship between baseline diastolic and systolic measures and memory performance on either of the two verbal-paired memory tests. Due to inconsistencies in the literature, it was

suggested that a relationship between blood pressure and memory may exist, but only for those who present an exaggerated reaction and/or poor recovery from the task. As predicted, memory performance was shown to be associated with both diastolic blood pressure reactivity and recovery for both memory tasks (when adjusting for age, education, medication and illness). When these two variables were entered into the regression model together, only poor recovery was independently associated with declarative memory performance. What is more, the effect of diastolic blood pressure recovery persisted when magnitude of task reactivity and task appraisals were taken into account; indicating that task appraisal is not a mediating factor between diastolic blood pressure and memory outcome. However, the relationship between diastolic blood pressure recovery and memory performance was only found for male participants, and there was no effect of systolic blood pressure on memory function for either men or women. It is believed that this is the first time a relationship between cardiovascular recovery and memory performance has been identified.

When interpreting this result one needs to take into account the effect of rumination on task recovery. Rumination is described as cognitions that focus attention on a negative event or negative mood (Rusting & Nolen-Hoeksema, 1998). Ruminating about a challenge could lead to later reactivation of the cardiovascular system, which may, in turn, account for poor cardiovascular recovery. For example, it has been reported that participants who were not given a distracter following a challenging task, had worse blood pressure recovery than those who were not given the chance to think about their performance. This relationship was also independent of original reactivity levels (Glynn et al, 2002; Suchday et al, 2004). In the present investigation a distracter was not used to control specifically for rumination. However, during the recovery period participants were engaged in a structured interview answering questions about

care-giving duties (responses from which were used in the larger study that this investigation was part of). From this it can be suggested that during the recovery period participants were suitably distracted. Therefore, the relationship between memory performance and recovery may not simply be due to rumination but more plausibly due to the effects of recovery from the task itself.

The possible pathogenic mechanisms underlying the influence of raised blood pressure on brain structure are uncertain, but some consideration of the pathways for the observed hypertension-memory association is needed. In a recent review, Waldstein (2003) outlines the possibilities for such a relationship, but her discussion is limited to the role of raised basal levels. Research has yet to discover what factors mediate the relationship between blood pressure responsivity (blood pressure reactivity and recovery) and memory performance. First, evidence has shown that hypertensives have smaller cerebral blood flow responses than normotensives during memory tasks (Jennings et al, 1998). This is important because adequate cerebral blood flow responses are needed for memory (and other cognitive functions). Structural changes to the blood vessels that are common in raised blood pressure decrease the blood supply to the brain. These include atherosclerosis in the large arteries and blockages in the smaller arterioles. In turn, these processes can lead to lesions of the white matter (portions of the brain involved in transmitting messages from one region to another) which detrimental effects on memory performance (Breteler et al, 1994; Palombo et al, 1997). Lastly, those with high blood pressure are also susceptible to brain atrophy which can result in memory decline. Once again, these suggestions are merely speculative, as neuroimaging data are not available with which to determine the prevalence of brain lesions or atrophy within the present sample. Furthermore, these points do not fully explain why a relationship should have been found between diastolic

blood pressure reactivity and declarative memory and not between diastolic blood pressure and cognitive reasoning ability as well.

Apart from discovering that poor diastolic blood pressure recovery is independently associated with poor memory performance in an elderly sample, two other interesting findings emerged. First, it is intriguing that diastolic but not systolic blood pressure was predictive of memory performance in this study. The explanation for this is unclear but it may simply be that a large number of systolic hypertensive subjects were eliminated through the screening procedure, or failed to respond to the study's invitation. Isolated systolic hypertension is a common problem in old age, so the selection procedure may have resulted in a group with relatively low systolic blood pressure. Second, it may be that the small arteries, which are influenced profoundly by diastolic blood pressure, undergo vascular atrophy progressively with age. In the presence of a stress-induced blood pressure increase this could be responsible for cognitive impairment (Hajdu et al, 1990). Several studies have shown that these hypertensive-related small vessel diseases may lead to the formation of white matter lesions in the subcortical area of the brain (Englund et al, 1988; Johansson, 1994). These lesions also have been associated with senile dementia (Blennow et al, 1991; Bots et al, 1993). So again, follow-up investigation of this sample's medical records for evidence of future clinical dementia would be valuable.

It is also of interest that the association between memory and diastolic blood pressure is only present in male participants. This could be explained in a number of ways. First, blood pressure and cognitive function are both affected by additional factors such as body mass index, smoking and exercise status. Table 2.1 indicates that these factors were no different for men and women, and therefore should not account for the gender differences observed in memory performance. Alcohol consumption,

which is known to exert an independent effect on both blood pressure (MacMahon, 1987) and cognitive function (Ryan & Butters, 1986) was significantly higher in men. However, when units of alcohol were controlled for, multiple regression analyses revealed that blood pressure reactivity and recovery were still positively associated with memory performance in male participants, thus ruling this explanation out. Second, the difference between male and female participants may be due to range restriction. Figure 2.5, shows that women had a smaller stress-induced diastolic blood pressure increase. This smaller response may have weakened the ability to detect associations between diastolic BP and memory performance among women. Therefore, in future a more challenging task may be needed for women to complete.

#### **2.4.4 Associations between Heart Rate and Memory Performance**

Relatively little work has investigated the relationship between heart rate and memory function in an elderly sample. Fauchaux et al (1983) suggested that heart rate recovery from a cognitive task may be worse for certain individuals, although they failed to relate heart rate to task outcome, while Backs and Ryan (1992) explore the notion that individual differences in autonomic activity may affect cognitive performance (mainly working memory). The present investigation aimed to explore whether a relationship between task-induced heart rate reactivity and declarative memory performance exists in an elderly sample, but also whether individual differences in recovery rates are relevant. Results revealed that there was no association between baseline heart rate and either of the two memory tasks, and no relationship was found between heart rate reactivity to the challenge and memory score on either of the tasks. The latter finding may be explained, however, by the fact that although care was taken to provide an adequate rest period before testing commenced, heart rate was still relatively high at baseline (70 bpm), and therefore did not increase sufficiently during

the task. Individual variability in heart rate recovery was present and this was significantly associated with memory performance on the first task, with ‘good recoverers’ correctly remembering 17 % more word-pairs than ‘poor recoverers’. This effect persisted when age, gender, education, illness, medication, magnitude of task reactivity and task appraisals were taken into account. The fact that statistical adjustment for task appraisals did not weaken the relationship between memory and heart rate recovery also implies that this relationship was independent of beliefs about the tasks. Interestingly, during the second memory task an interaction between memory score and gender was present (when entered competitively into the regression model with heart rate recovery). *Post hoc* analyses revealed that this was due to an association between memory performance and heart rate recovery in female participants.

In summary, it is believed that this is the first time individual rates of heart rate recovery have been positively associated with memory performance in an elderly sample. The present results also question the notion that this relationship is modified by motivational factors, or is a result of variation in the magnitude of heart rate increase in response to the challenge. However, little is known yet about the precise mechanism by which heart rate may detrimentally affect memory performance. One might speculate that changes in sympathetic activity (marked by heart rate increases) could affect cognitive processing areas of the brain much in the same way that glucocorticoids and blood pressure are said to. Neuroimages of the sample, or follow-up of medical records for incidence of future dementia would be of great value. In the mean time the findings need replication to be truly credible.

## **2.4.5 Associations between Heart Rate Variability, Pre-Ejection**

### **Period and Memory Performance**

This particular investigation failed to detect any association between baseline heart rate variability and declarative memory or cognitive reasoning function. What is more, no relationship was present between cognitive function and heart rate variability reactivity or recovery. Similar null results were discovered for pre-ejection period levels. One explanation for these findings may relate to survival effects that result from selection attrition. That is, individuals may be excluded from the elderly sample because of cardiovascular morbidity, or they may be unavailable for participation due to cardiovascular mortality. Therefore, participants might not be producing strong enough heart rate variability / pre-ejection period responses from which to detect task-induced alterations. This is suggested by the weak reaction elicited by the cognitive tasks and could be explained by the relatively good health status of the sample. Longitudinal data are needed to fully describe the age-related effects of heart rate variability / pre-ejection period on cognitive performance. Because a relationship was discovered between the other cardiovascular factors and memory, the null results seem to be explained by the sample's weak reaction to the task. It could be suggested that the tasks used in this particular investigation were not strong enough to detect subtle changes in pre-ejection period and heart rate variability, thus masking an actual association between these cardiovascular functions and cognitive performance. Future investigation would need to repeat this procedure with a more effective challenge.



## 2.4.6 Associations between Cardiovascular, Neuroendocrine

### Responsivity and Memory

The stress response is associated with activation of the HPA axis and autonomic nervous system. In the present investigation cortisol was used as a marker of neuroendocrine activity, while blood pressure and heart rate were used to represent cardiovascular system activation to challenge. Results have shown that activation of both these systems by a challenging event was related to a decline in declarative memory performance. The literature is unclear as to whether both these systems are simultaneously or independently associated with memory function during a challenging situation. Results reveal for the first task (VPA 1) that only heart rate recovery was significantly related to declarative memory performance. However, by the second task (VPA 2) both cortisol reactivity and heart rate recovery were independently associated with memory performance. The association persisted when age, gender, education, illness, medication, magnitude of baseline levels and task appraisals were accounted for. The fact that associations with cortisol were not significant in the analysis of VPA 1 involving heart rate is important, although the effect was not far off significance ( $p = .11$ ). One reason for this may lie in the task order. Since cortisol typically responds to challenge less rapidly than does the cardiovascular system, the impact of cortisol responsivity may be more apparent with tasks later in a session. Hence cardiovascular recovery was relevant to VPA 1, while both cardiovascular and neuroendocrine responses were related to VPA 2. Dual analyses of blood pressure recovery and cortisol responsivity reveal that for the first and second memory task blood pressure recovery and cortisol responsivity both independently predicted memory function.

### **2.4.7 Section Summary**

Overall, the results suggest that individual task-related diastolic blood pressure, heart rate and cortisol responsivity were associated with memory performance. These findings may be considered within the broader context of allostasis. Allostasis is defined as the body's ability to adapt its internal physiologic setting to match external demand (McEwen & Stellar, 1993). However, when there is a cumulative build-up of physiologic strain, dysregulation across multiple systems leading to functional decline can occur. This idea of bodily 'wear and tear', known as allostatic load, is thought to increase over time, and is therefore common in elderly populations. Seeman et al (1997b) found that higher allostatic load scores were associated with poorer cognitive performance and physical functioning among elderly participants, based on ten parameters reflecting levels of physiologic activity across a range of important regulatory systems (including markers of neuroendocrine and cardiovascular function). Karamangla et al (2002) discovered that baseline allostatic load also predicted changes in physical and cognitive functioning over a 7 year follow-up period. In these particular studies the overall construct of allostatic load was considered, rather than its separate components. It is also possible to assess individual elements of allostatic state (or determine individual allostatic state) by investigating the response profiles of primary mediators involved in the responsivity process (for example, cardiovascular and neuroendocrine response). One important response profile of allostatic state is a prolonged response or, the delayed shut down of the mediator after exposure to a stressor. Results for this particular investigation show that those participants with poor heart rate and diastolic blood pressure recovery presented poorer memory functioning following a cognitive challenge. The individual variability in recovery rate and related cognitive performance suggests that some participants are more resilient than others to

the effects of the task. Resilience is an example of successful allostasis in which ‘wear and tear’ is minimised. Thus, instead of suggesting that prolonged recovery or greater evidence of allostatic load is related to cognitive decline, it can be postulated in turn that resilience to stress or successful heart rate and diastolic blood pressure recovery are in fact associated with effective memory function among an elderly sample. However, it has yet to be determined whether this association is due to the absence of a direct effect of cardiovascular dysregulation on brain function or whether it is simply the case that functioning well in old age is synonymous with good memory and good psychobiological function (i.e. rapid recovery from stress).

#### **2.4.8 Limitations of the Current Investigation**

The present study revealed that both stress-induced cardiovascular and neuroendocrine responses are associated with declarative memory performance. In doing so, the investigation used performance-based tests rather than self-report methods to assess cognitive function. The investigation also enforced strict control of demographic characteristics and motivational factors and employed an adequate sample size of both male and female elderly participants. The selection criteria and controls employed in this analysis significantly reduce the possibility that functional decline may be due to an undesirable side-effect of medications used to treat diabetes, hypertension or cardiovascular disease. The findings that neuroendocrine and cardiovascular factors may be associated with functional disturbance over and above cardiovascular risk factors and independent of cardiovascular disease clearly indicated that disturbances in cognitive ability in elderly men and women cannot be attributed solely to cardiovascular disease, and that alterations in neuroendocrine and cardiovascular activity represent additional independent sources of risk. Careful selection of more than one cognitive test of sufficient sensitivity added credibility to the findings. Results also show that

associations were found between physiological mechanisms and memory and not cognitive reasoning ability; although Beglinger et al (2000) suggest that this test is sensitive (and rated relevant) among an elderly sample.

Nevertheless, various limitations to the study must be considered. First, previous investigations have evaluated cortisol reactions to stressful tasks, and then administered memory tests, showing that memory performance is inversely related to cortisol stress reactivity (Kirschbaum et al, 1996; Lupien et al, 1997; Wolf et al, 2001b). The current design was different in that cognitive performance was associated with cortisol and cardiovascular responses to these same memory and reasoning tasks. One limitation of this is that this single purpose study may have had a double effect. That is, it is not entirely clear whether task response was directly associated with task performance or whether individuals who performed more poorly were simply more stressed. An attempt was made to address this concern by controlling statistically for subjective task appraisals. Monitoring beliefs about tasks performance, involvement, stress and difficulty did not appear to mediate the relationship between physiological responsivity and memory performance. However, although monitoring of task appraisals was beneficial it has to be conceded that these may not have provided a complete picture with regard to this issue. Clearly under these circumstances, no causal conclusions can be drawn. It is not possible to infer that physiological dysregulation causes memory disturbances, that poor memory performance elicits physiological dysregulation, or that both effects are due to a third common determinant. And as a result of the design employed in this investigation it is also difficult to make direct comparisons with other studies in this area. In future it would be advisable to administer a stress task followed by a separate memory assessment, however, because

the study was conducted as part of a much larger investigation on an elderly population it was not deemed appropriate in these circumstances.

Second, a general practice framework was used for sampling to reduce healthy volunteer bias. The study may nevertheless have attracted volunteers interested in issues surrounding health and ageing, so results may not be typical of the wider population. The study was also limited to older adults of white European origin and may not generalise to other ethnic groups. More importantly, because the response rate was only 25 % it is not known how many of those who did not take part actively refused, or failed to respond to the invitation to participate. It is certainly possible that participation was weighted towards higher functioning individuals (as it was noticeable that the number of chronic illnesses averaged 1.3). This means that the true extent of the relationship between physiological responsivity and cognitive performance could have been attenuated.

Third, it is notable that participants experienced a small magnitude of cardiovascular and cortisol response. This may have been due to the particular challenges that were administered. Although not designed specifically to induce psychological stress, the results show that the cognitive challenge did produce sufficient physiological stress along with providing a suitable cognitive challenge. However, this also meant that the true extent of some of the findings may have been masked.

Fourth, due to sample limitations, the test session was not sufficiently long to measure a recovery phase for cortisol production, and both cortisol responsivity and recovery could not be assessed. The majority of studies in the literature examining physiological responsivity to challenge have focused primarily on individual response amplitude in younger samples (Kirschbaum et al, 1996; Wolf et al, 2001b; Domes et al, 2002). However, work with rats has shown that ageing has little effect on the amplitude

of the initial cortisol response. Instead it is associated with post-stress recovery to basal levels (Sapolsky et al, 1986; Odio & Brodish, 1989). The delayed termination of neuroendocrine activity following stress is thought to reflect deficits in glucocorticoid feedback regulation (De Kloet, 1991). This suggests that the association shown between cortisol responsivity and memory may have been more pronounced had cortisol recovery and cognition been tested. However, the generalisability of the above findings in rodents to humans remains unclear, as there is evidence of both support (Seeman & Robbins, 1994) and rejection of this hypothesis (Nicolson et al, 1997). It is important therefore that future research with an elderly population endeavours to explore the relationship between cognition and cortisol recovery.

Fifth, limitations surrounding blood pressure measurement exist. During the investigation heart rate was continuously assessed; however, due to an oversight in development of the experimental protocol no blood pressure reading was taken after task three (VPA 2). Blood pressure was also not measured during the tasks themselves. This creates two limitations. First, because only two blood pressure readings were taken this meant that a task average was not possible. A task average would have been more robust and more desirable because using the task maximum increases the chance that the sole blood pressure reading may have been due to factors other than the task itself. Second, it would also have been advantageous to continuously monitor blood pressure throughout the tasks. By using a continuous monitoring device such as the Portapres system blood pressure responses during the tasks could have been ascertained more reliably in relation to task performance. Assessment of blood pressure responses after completion of the tasks also makes determining the full extent of cardiovascular response to the cognitive challenges very difficult, as blood pressure responses were

almost certainly underestimated. As a result it can only be inferred that elevated blood pressure following the two cognitive tasks was related to memory performance.

Sixth, the cross-sectional design only allowed examination of cognitive performance at one point in time, therefore the causal sequence of events is not known. The results are consistent with the notion that heightened cortisol responsivity and delayed cardiovascular recovery produce impairments in memory function. However, one can only be speculated that these effects occur over time. Although longitudinal research points to a cumulative contribution of glucocorticoids on memory function (Lupien et al, 1995; Lupien et al, 2002), acute effects of cortisol on cognitive functioning, possibly due to alterations in calcium influx, must be considered as well (Joëls & De Kloet, 1994; Joëls, 2000). It is also possible that some third factor (such as underlying dysfunction in the central nervous system) could cause both the poor memory performance and alterations in stress-related physiology. In addition, only cognitive function in an acute experiment on a single occasion was assessed. Although some evidence exists to suggest an association between distress proneness and long-term cognitive decline (Wilson et al, 2005), at the moment one can only speculate that this brief snapshot of stress responsivity is how participants would typically react to anxiety provoking situations throughout life. Correspondingly, mild deficits in memory performance and increased cortisol, blood pressure and heart rate responsivity are both synonymous with old age. One therefore needs to employ a longitudinal design with brain imaging to determine whether cardiovascular and neuroendocrine responsivity are associated with memory deficits over time. Recent large population-based longitudinal studies have shown that increased cortisol response was associated with cognitive decline two years later (Kalmijn et al, 1998), and in patients with Alzheimer's disease, increased cortisol response was associated with cognitive decline during the following

year (Swanick et al, 1996) and with increased risk of death over four years (Miller et al, 1994).

Seventh, the results of the present investigation appear to suggest that cardiovascular and neuroendocrine responsivity are associated with memory performance but not cognitive reasoning ability. Although it has been theorised that this may be the result of physiological effects on the hippocampus-driven declarative memory, it also has to be acknowledged that these effects may simply be due to task selection. That is, the theoretical conclusions of the study which draw on the division between hippocampal and non-hippocampal cognitive performance may be due to the fact that only one cognitive task (for each category) were applied on one occasion. Replication of the study utilising different tasks is therefore needed in order to support current interpretations.

Lastly, although, one can tentatively conclude that the neuroendocrine and cardiovascular system both influence memory performance, replication of the study needs to include brain regions other than the hippocampus and neuroendocrine and cardiovascular compounds, such as adrenaline, noradrenaline, corticotrophin releasing hormone, ACTH and proinflammatory cytokine concentrations (Lupien & Lepage, 2001). Evidence suggests that corticotrophin releasing hormone is known to influence memory retrieval (Rooszendaal et al, 2002; Gulpinar & Yegen, 2004); however, because of the difficulty involved with measuring human CRH levels, it is not clear whether the associations observed between cortisol and memory performance could be a result of CHR elevation as well. Future investigation would need to assess other components of the autonomic nervous system and HPA axis to be certain.



## **2.4.9 Implications for Future Research**

Those over the age of 65 represent one of the fastest growing sections of the population. Within an older population approximately 50 % will complain of cognitive deficits (including poor memory). It is the integrity of these cognitive abilities which are vital for the elderly to maintain their independence and a sufficient quality of life (Van Boxtel et al, 1997; Waldstein, 2003). It was argued in this investigation that not all individuals age at the same rate, and the results may have provided an insight into one reason why this may be so. Understanding the factors which contribute to differential risks for cognitive decline is important to patients and clinicians alike. That is, the findings of this investigation will only be truly valuable if they are applied to prevention or treatment strategies. The evidence for this is so far encouraging. Muldoon et al (2002) in a randomised, double-blind treatment, cross-over trial of six antihypertensive medications showed favourable effects for six treatments designed to reduce blood pressure and increase working memory performance.

In contrast, most trials investigating raised cortisol levels and increased memory performance have been conducted with Cushing's disease patients, not elderly samples (Starkman et al, 1992; Starkman et al, 2003). However, a recent study by Lupien et al (2002) found that memory performance in two groups of elderly participants (separated on the basis of their cortisol history over a 5 year period) could be modulated by a metyrapone-hydrocortisone, hormone replacement protocol. Metyrapone works by blocking 11 $\beta$ -hydroxylase and thus the conversion of 11-desoxycortisol to cortisol. Lupien et al (2002) suggest that long term treatment with metyrapone could lead to beneficial effects on memory performance in the high cortisol group. Such positive effects of long-term treatment with substances which reduce cortisol levels have been obtained in populations suffering from depression and Cushing's disease-related

hypercortisolism (Checkley et al, 1997; Starkman et al, 1999). Further work on memory performance, neuroendocrine reactivity levels and dose dependent glucocorticoid manipulation is needed in order to investigate the level at which pharmacological intervention could be beneficial to elderly participants.

Research has also focused on multidisciplinary approaches to explore memory improvement in old age. Rapp et al (2002) conducted a clinical trial testing the efficacy of cognitive and behavioural treatment to improve memory performance and participant's attitudes about their memory. The multifaceted intervention which included education, relaxation training, memory skills training and cognitive restructuring for memory-related beliefs, found that individuals with mild cognitive impairment did benefit from memory enhancement training. Future research needs to concentrate on examining effective pharmaceutical and stress-reducing therapies for those individuals whose memory is affected by stress-induced cardiovascular and neuroendocrine responsivity. Further understanding of the relationship between blood pressure, cortisol, heart rate and memory function is critical to the development of prevention / treatment strategies and interventions centred on preserving cognitive functioning in the elderly.

#### **2.4.10 Conclusion**

Despite the limitations outlined earlier, the results of this study reliably demonstrate that activation of the neuroendocrine (marked by cortisol reactivity) and cardiovascular systems (marked by poor blood pressure and heart rate recovery) in certain elderly individuals during a challenging event is significantly associated with declarative memory, but not cognitive reasoning performance. What is more, it appears that individual variability in neuroendocrine and cardiovascular responsivity affect memory performance simultaneously. Such heterogeneous findings would suggest that

ageing may not be associated with uniform changes in neuroendocrine and cardiovascular function. It is therefore speculated that those elderly individuals classed as ‘high responders’ could be exposed to a life-long accumulation of raised neuroendocrine or cardiovascular levels during times of stress, thus resulting in a build-up of physiological strain and dysregulation across multiple systems from which functional decline can occur. This bodily ‘wear and tear’ or allostatic load may build over time and therefore be more common in elderly populations (Seeman et al, 1997b; Karlamangla et al, 2002). Although it is accepted that other factors (such as environment and diet) could influence this process as well, the individual variability in recovery rate and related cognitive performance for this sample suggests that some participants were more resilient than others. Physiological resilience, manifested in lower cortisol responses and more rapid post-task cardiovascular recovery, appears to be associated with more effective memory function among older people. The integrity of cognitive abilities is vital for the elderly to maintain their independence and quality of life. Understanding psychobiological responsivity may increase insight into risks for differential decline in cognitive abilities in old age. Further research now needs to replicate and extend these findings in order to advance our understanding of the underlying mechanisms between individual physiological responsivity and memory decline and preventative and treatment strategies which could improve these deficits.

## **Chapter 3: Subjective Socioeconomic Position, Gender and the Cortisol Response to Awakening in an Elderly Population (Study 2)**

### **3.1 Introduction**

#### **3.1.1 Rationale**

Allostatic load is believed to occur as a result of repeated physiological strain over the life course. When the body receives significant challenge over many years, physiological systems may cease to function effectively resulting in physical, as well as cognitive impairment (as outlined in Study 1). However, because there is great variability in physiological functioning in old age (Crimmins et al, 2003), it is important to explore the factors which leave some individuals more vulnerable to physiological dysregulation and allostatic load in later life. It is important therefore to understand more comprehensively the psychobiological mechanisms by which various aspects of life impact collectively on health. One important consideration is the influence of socioeconomic factors. Study 2 aims to investigate the relationship between socioeconomic status and neuroendocrine dysregulation in later life.

#### **3.1.2 Socioeconomic Status and Health**

Socioeconomic status (SES) is a reflection of social position, typically encompassing income, education and occupation (Adler & Snibbe, 2003). The inverse relationship between socioeconomic position and health is well established, with individuals at the highest socioeconomic level enjoying better health than not only those

at the bottom of the social scale but at all levels in between (Marmot et al, 1991; Marmot & Shipley, 1996). Lower socioeconomic status has been associated with biological and behavioural risk factors, all cause mortality and a wide range of diseases, including heart disease, gastro-intestinal disease and respiratory disorders (Adler & Ostrove, 1999; Steptoe & Marmot, 2002). A relatively novel hypothesis is that these effects are due to chronic stress which is associated with socioeconomic status.

### ***3.1.2.1 Socioeconomic Status, Chronic Stress and Age***

Lower socioeconomic status is reliably associated with a number of important social and environmental conditions that contribute to chronic stress burden. For example, it has been shown that individuals from lower socioeconomic groups report less effective coping, greater exposure to stressful life events and a greater impact of these events than individuals with higher socioeconomic status (Anderson & Armstead, 1995; Lachman & Weaver, 1998; Bosma et al, 1999). This suggests that individuals from lower socioeconomic groups may have increased vulnerability and exposure to stress, and subsequently increased disease as well (McEwen & Wingfield, 2003b). Related to chronic stress is the concept of allostatic load. This model specifies that repeated wear and tear on the body is associated with repeated or prolonged activation of stress systems which occur when one is frequently exposed to a stressor (Baum & Posluszny, 1999). As one gets older the frequency of exposure and consequences of stress will increase. This suggests that the effects of socioeconomic status on health will be particularly marked in old age. A lifetime of economic or social hardship has been found to promote earlier decline in physical, psychological and cognitive functioning, and increased mortality (Lynch et al, 1997). The cumulative impact of socioeconomic strain over many years may, therefore, leave some individuals more vulnerable to ill health in old age. However, the mechanisms through which these

health inequalities influence risk factors and disease incidence are still poorly understood.

Lifestyle factors and access to health care contribute, but the gradient in mortality and biological risk persists after smoking, lack of physical exercise, alcohol consumption and other health behaviours are taken into account (Adler et al, 1994a; Lantz et al, 2001; Steenland et al, 2002). Research has now begun to look at the relationship between physiological changes, socioeconomic status and stress. To date, higher socioeconomic status has been associated with better cardiovascular function (Gump et al, 1999); however few studies have examined the effect of other physiological pathways. A psychobiological pathway, where low socioeconomic status elicits sustained activation of neuroendocrine responses, in turn promoting the development of pathology, is proposed (McEwen & Seeman, 1999; Steptoe & Marmot, 2002). Cortisol may be an important indicator, since it is implicated in metabolic syndrome, type II diabetes, abdominal adiposity, depression and other conditions related to socioeconomic status differences. Although short-term glucocorticoid response to stressful situations serves as an adaptive function, chronic exposure to elevated cortisol concentrations eventually contributes to HPA dysregulation and allostatic load (McEwen & Seeman, 1999; Brown et al, 2004).

### **3.1.3 Methodological Design Considerations**

Two methods are commonly used to investigate these health-related psychobiological pathways: laboratory based studies assessing acute physiological response to stressful stimuli and naturalistic monitoring of neuroendocrine functioning in everyday life. Although laboratory studies allow for stringent control, they are limited by the fact that they measure acute responses to a brief stimulus at one point in time. With the advent of salivary assays, naturalistic studies have increased due to their

non-invasive nature and ease of sample collection by research participants at various points during their daily routine. Naturalistic studies employing salivary cortisol sampling therefore allow the influence of everyday experiences on specific biological processes to be repeatedly assessed without the interruption or disturbance of normal behavioural patterns. To date few studies have employed these techniques when investigating the impact of socioeconomic status on physiological functioning.

A key factor in disease-associated physiological mechanisms is the cortisol awakening response (CAR). Activity of the hypothalamic-pituitary-adrenal axis is characterised by a robust circadian rhythm, whereby cortisol increases by 50-100 % within the first 20-45 minutes of waking (Pruessner et al, 1997). Cortisol is of major importance with regard to stimulation response, subsequently the cortisol awakening response is sensitive to situational factors such as weekday or weekend (Schlotz et al, 2004; Kunz-Ebrecht et al, 2004a) and time of waking (Backhaus et al, 2004; Williams et al, 2005), but is also increased in people reporting high levels of general stress or work stress (Schulz et al, 1998; Wüst et al, 2000; Pruessner et al, 2003). A reduced awakening response has been observed in burnout, chronic fatigue and in people reporting health problems (Pruessner et al, 1999; Kudielka & Kirschbaum, 2003; Roberts et al, 2004). The main purpose of this study was to discover whether low socioeconomic status is associated with a heightened cortisol awakening response in old age.

### **3.1.4 Socioeconomic Status and the Cortisol Awakening Response**

The use of the awakening response to study socioeconomic status inequalities in biological regulation and allostatic load has been relatively limited thus far. An early study demonstrated that cortisol samples between 07:00 h and 08:00 h were positively associated with socioeconomic status measured by education or occupation

(Brandtstdter & Rothermund, 1994). Recent studies have also shown that among females, high financial strain was related to higher morning cortisol levels (Grossi et al, 2001) and children with low socioeconomic status present significantly higher morning salivary cortisol levels than children with high socioeconomic status (Lupien et al, 2001). Similarly, an investigation of salivary cortisol in children aged 6-10 years found an inverse association with parental socioeconomic status and waking cortisol level (Lupien et al, 2000). However, neither of these studies assessed the cortisol awakening response itself. Four recent studies investigating this issue have provided rather inconsistent evidence. The first three studies, using the same sub-sample of the Whitehall II epidemiological cohort, found that cortisol response to awakening on a working day did not differ by socioeconomic status; but, that waking cortisol response was greatest in women and for those from the low social status group who reported higher job demands (Steptoe et al, 2003a; Kunz-Ebrecht et al, 2004a; Kunz-Ebrecht et al, 2004b). It has also been shown that in comparison with lower educated white-Americans lower educated African-American participants produced a steeper cortisol awakening response (Bennett et al, 2004).

Studies in the area were carried out primarily on individuals of working age. A difficulty with studying people of this age is that lower socioeconomic status individuals typically experience greater work stress than more privileged groups. It is not clear therefore which factor is related to a heightened cortisol awakening response. With an increasing proportion of the population at retirement age research now needs to investigate socioeconomic status and cortisol response to awakening in individuals who are not part of the workforce (Adler & Snibbe, 2003). The present study assessed the association between socioeconomic status and the cortisol awakening response in an older retired population that was not part of the work force.



### **3.1.5 Measurement of Socioeconomic Status**

Indicators of socioeconomic status have ranged from education levels, occupation and total family income to more complex tools such as adequacy of financial resources, number of people living on family income, housing standards and neighbourhood issues (Kneipp & Drevdahl, 2003). However the measurement of socioeconomic status in old age is complicated since conventional markers such as current occupation are not applicable. Current income does not reflect lifetime differentials, while education is usually completed early in life, so may be less predictive in later years (Davey Smith et al, 1998). Additionally, Wilkinson (1999) has argued that it is not absolute level of socioeconomic status that is important, but rather perceptions of status based on relative inequality. An alternative approach is to use subjective social status as an indicator. Adler et al (1999) devised a simple, one-item measure for assessing people's perceptions of their position within the social hierarchy. Subjective social identity is a complex phenomenon; a measure of subjective social status is likely to reflect not only current social circumstances but also incorporate an assessment of the individual's past (socioeconomic, educational and economic background), along with their future prospects. In a study of healthy women, it was found that subjective social status was more consistently related to factors such as self-rated health, body fat distribution and cortisol habituation to repeated stress than were objective socioeconomic status measures. The higher women placed themselves on the ladder, the lower was their chronic stress, subjective stress, negative affect, pessimism, and passive coping and greater was their perceived control over life and active coping (Adler et al, 2000). Singh-Manoux et al (2003) also measured subjective social status in the Whitehall II cohort, and found that only 49 % of the variance was accounted for by objective indicators such as employment grade, education, material deprivation and

household income. Subjective social status was inversely associated with the presence of angina, diabetes, respiratory illness and self-rated health. This measure of subjective social status had also been associated with self-reported global health after controlling for objective measures of socioeconomic status, and has been found to demonstrate good test-retest reliability over a six month period (Operario et al, 2004).

In the present study, subjective social status was used as an indicator of chronic strain in order to determine its effects on neuroendocrine dysfunction. To achieve this, salivary cortisol was assessed five times in the hour after waking in elderly individuals who were classified according to the subjective social status scale. It was hypothesised that the cortisol awakening response would be heightened in people of lower social status. Smoking, time of waking and body mass index can influence cortisol so these factors were included as covariates (Clow et al, 2004). Additionally, for the presence of chronic illness and prescription of chronic medication were adjusted for statistically, since Kudielka and Kirschbaum (2003) reported that the cortisol awakening response is smaller in people with physical illnesses.

### **3.1.6 Gender, Socioeconomic Status and the Cortisol Awakening Response**

Another issue that was explored in this study was gender differences in the cortisol awakening response. These have been inconsistent in younger samples, with some studies showing greater or more sustained cortisol awakening response in women than men (Pruessner et al, 1997; Pruessner et al, 1999; Kunz-Ebrecht et al, 2004b), while others have reported no gender differences (Edwards et al, 2001; Kudielka & Kirschbaum, 2003). A recent meta-analysis of cortisol responses to challenge has

shown greater age-related increases in response in women than men, suggesting that gender differences might be accentuated in older age (Otte et al, 2005).

### **3.1.7 Timing of Cortisol Awakening Response**

Measurement of the cortisol response to waking is typically calculated by assessing the total amount of cortisol produced (using area under the curve), or by subtracting the peak concentration from the concentration on waking to gain a response level. Although both methods are deemed reliable, there is still little consensus on the timing of saliva sample collection (Clow et al, 2004). This is illustrated by the variety of time points measured in the studies reviewed for this investigation. For instance, the Whitehall II sample took cortisol samples on waking, and then at regular 30 minute intervals after waking (Kunz-Ebrecht et al, 2004a; 2004b; Steptoe et al, 2004). In contrast, other studies assessed salivary cortisol on waking and then either just 45 minutes after waking (Broderick et al, 2004) or 30 and 45 minutes after waking (Schlotz et al, 2004; Federenko et al, 2004). One study however, chose to sample saliva on just one occasion, immediately after waking (Brody et al, 2000).

The problem with sampling saliva on only two occasions, or with a long delay after waking (>30 min) is that there is no consistent evidence to indicate when the post-waking peak in cortisol will occur, or whether this peak will be at the same time for all groups being tested. It seems logical therefore, that salivary cortisol samples should be assessed at regular short intervals after waking. This has already been achieved by some authors who assessed post-waking cortisol samples at 15 minute intervals up to one hour after waking (Schulz et al, 1998; Kudielka et al, 2003). In order to allow for the uncertainty which surrounds the timing of peak morning cortisol elevations, the present study will assess saliva samples at 10, 20, 30 and 60 minutes after waking.

### 3.1.8 Compliance and Salivary Cortisol Sampling

With the move towards collection of data in the natural environment, factors such as sample compliance need to be considered. Non-compliance with the sampling schedule is a potentially confounding factor that needs to be taken into account. Saliva sampling is non-invasive and easy to apply to naturalistic studies, but it is difficult to control sample adherence and timing. In a recent study, it was demonstrated using electronic timing devices that a measurable proportion of individuals did not take samples as required (Kudielka et al, 2003). In another study, 93 % of participants reported that they had adhered to the sampling schedule, however with use of an electronic timing device it was shown that only 71 % complied with timing instructions (Broderick et al, 2004). It has previously been demonstrated by Professor Steptoe's group, that participants who delay more than 10 minutes between waking and taking the first sample show almost no cortisol awakening response (Kunz-Ebrecht et al, 2004a). Failure to adhere to timing has serious implications for analysis of the cortisol samples, and may lead to a false blunting of the cortisol awakening response. In the present study participants were asked to keep a diary record of each sample in order to determine if delay in collection had occurred. As a result, it was additionally hypothesised that individuals who reported delays of more than 10 minutes between waking and taking the first sample would have higher 'waking' values and a smaller cortisol awakening response than would compliant participants. The findings of this study have been published in *Psychoneuroendocrinology* (please refer to reprints at the end of the thesis).

## **3.2 Method**

### **3.2.1 Participants**

This study used the same sample of older adults employed in Study 1. One hundred and thirty nine men and women aged 65 to 80 years were recruited by letter from two general practices in the London area for a study of ageing and health (Appendix I). Patient databases were searched for individuals who were dwelling in the community and had no record of coronary heart disease, tachycardia, aortic valve regurgitation, dementia, psychosis, and no cancer evident in the last five years. One hundred and thirty three individuals completed a clinical assessment session described in Section 2.2.5 of whom 112 (84 %) agreed to take cortisol samples over the period after waking. Sampling was incomplete from 9 individuals because of insufficient saliva or failure to take all samples. Of the 103 who did carry out saliva sampling over the hour after waking, 10 were excluded from these analyses since they were taking oral corticosteroid medication. As detailed in section 3.2.5, 10 individuals did not adhere to the sampling schedule, and data concerning subjective social status were missing from a further two participants. The primary analyses were therefore conducted on 81 participants (40 men, 41 women). There were no significant differences between these 81 participants and the remaining 52 who completed the clinical assessment in terms of age, gender distribution, subjective social status, and marital status, number of chronic illnesses, medication count, or time of waking in the morning. Informed consent was gained from each individual and ethical approval was granted by Camden and Islington Local Research Ethics Committee (Appendix III).

### **3.2.2 Cortisol Measures and Procedure**

Participants who completed the clinical assessment session described in Study 1 (Section 2.2.5) were asked whether they would collect saliva samples at home as well. They were requested to take samples immediately on waking, and then at 10, 20, 30 and 60 minutes after waking. All participants had practised using Salivettes (Sarstedt, Inc., Leicester, UK) during the clinic session, and were instructed to take the first sample whilst lying in bed and not to brush their teeth, eat, drink or smoke before the fourth sample. Otherwise, they were free to follow their normal daily routines. Participants stored the saliva samples in domestic refrigerators until they were sent to the laboratory. They were subsequently stored in a laboratory freezer until they were thawed for the biochemical analysis using a time-resolved immunoassay with fluorescence detection (Dressendörfer et al, 1992). In addition to the saliva sampling, participants recorded when they woke up and exactly when each sample was taken. They also gave a simple rating of whether their sleep quality had been good or poor on the previous night (Appendix XIII).

### **3.2.3 Measures of Social Status**

Before the clinical assessment session, a questionnaire was completed from which a number of measures were included in this analysis.

#### **3.2.3.1 Subjective Social Status**

Subjective social status was assessed with the “ladder” measure (Adler et al, 2000). Participants were shown a drawing of a ladder with 10 rungs, representing where people stand in society (Appendix XIV). They were told that at the top of the ladder were the people who are best off, those who have the most money, most education, and best jobs. At the bottom were the people who are the worst off, have the

least money, least education and the worst jobs or no job. They were asked to place themselves on the rung which they felt they stood.

### **3.2.3.2 Objective Social Status**

Educational background was assessed as an objective correlate of socioeconomic status. Participants were asked about their qualifications, and what age they had completed their education. They were divided according to whether they had any educational qualifications (from high school certificates upwards) or no qualifications. Financial strain was measured as an additional indicator of socioeconomic status, since it is typically greater in lower socioeconomic status individuals (Marmot et al, 1991). This was assessed by averaging two 4-point ratings, asking participants how satisfied they were with their financial situation (Appendix XV). Higher scores indicated greater financial strain. In subsequent analyses, the sample was divided by binary split into lower and higher financial strain groups.

### **3.2.4 Other Measures**

Depression was measured using the 20-item Centre for Epidemiologic Studies-Depression Scale (CES-D) (Radloff, 1977). High scores indicate greater depression. Optimism was assessed using the Life Orientation test (LOT) (Scheier & Carver, 1985), with higher scores indicating greater dispositional optimism (Appendix XVI).

Chronic illness and medication were assessed with indices similar to those used in Study 1 and by other investigators (Benyamini et al, 2004; Seeman et al, 2004). A list of chronic illness was assembled from medical notes and patients' reports. The number of serious medical conditions (e.g. diabetes, arthritis, hypertension) was summed. Medication was assessed by summing the number of classes of long-term

medication, including antihypertensives, statins, and anti-inflammatory medications (Appendix VIII).

Smoking was assessed by enquiring whether participants smoked, and (if they were former smokers) when they had stopped smoking. A division was made between non-smokers, and those who were either current smokers or had stopped within the past 5 years. Alcohol consumption was assessed by asking people if they drank alcohol, and how many measures they drank in the average week. Responses were divided according to whether the respondent drank more or less than five measures in the week. Habitual physical activity was categorised according to whether the person walked briskly for at least 20 minutes more than twice a week, once or twice a week, or never (Appendix IX). Anthropometric measures were obtained during the clinical assessment session, from which body mass index and waist to hip ratio were calculated.

### **3.2.5 Statistical Analysis**

Comparisons of background characteristics were made using analysis of variance and nonparametric statistics as appropriate for continuous and categorical variables, respectively. Non-compliant participants were defined as those who reported a delay of more than ten minutes between waking and taking the first saliva sample. The impact of compliance on the cortisol awakening response was tested using repeated measures analysis of variance of the five cortisol samples, with compliance status as the between-subject factor. The Greenhouse-Geisser correction for degrees of freedom was applied when the assumption of sphericity was violated, and *p* values were adjusted accordingly.

The main analyses involved comparison of higher and lower subjective social status groups, defined by a cut-point of five on the subjective status measure. After exclusion of non-adherent participants, repeated measures analysis of variance was



carried out on the five cortisol samples, with subjective social status (higher, lower), and gender as between-subject factors. The cortisol awakening response was defined as the change between waking and 30 minutes and was compared across groups using analysis of covariance, with body mass index, waist to hip ratio, smoking status and time of waking as covariates. In order to determine whether the influence of subjective social status was independent of objective socioeconomic status, educational attainment and financial strain were included as additional covariates. The number of chronic illnesses and medication count were also added as covariates, to discover whether social status and gender effects on the cortisol awakening response were independent of illness status as well. Two further repeated measures analyses of variance were carried out on the five cortisol samples, with the study group divided on the basis of educational attainment and financial strain, so as to assess the impact of objective socioeconomic status. Data are presented as means  $\pm$  standard deviations.

### 3.3 Results

Table and Figures to accompany this section can be found from page 168.

#### 3.3.1 Characteristics of Higher and Lower Social Status Groups

Details of the higher and lower social status groups are provided in Table 3.1.

The proportion of men and women in the two groups did not differ, nor were there differences in age, marital status, or in number of chronic illnesses or medications prescribed. There was no difference in men and women's subjective social status scores ( $p = .41$ ) which averaged  $5.88 \pm 1.7$  and  $5.60 \pm 1.3$ , respectively. Higher social status participants had left school at an older age on average ( $F_{1,77} = 9.66$ ,  $p = .003$ ), and were more likely to have educational qualifications than the lower social status participants

( $\chi^2 = 6.82$ ,  $p = .009$ ). Lower social status participants experienced marginally greater financial strain ( $F_{1,76} = 3.89$ ,  $p = .052$ ). There were no significant differences in body mass index, waist to hip ratio, or in health behaviours (smoking, walking and alcohol consumption) between the two groups. One quarter of participants indicated that their sleep had been poor on the night preceding saliva sampling, but this proportion did not vary with social status. There were no group differences in depression or optimism as assessed with the LOT and CES-D. The higher and lower social status groups did not differ in the time of waking on the sampling day. Overall, participants woke up at 06:51 am  $\pm$  57 minutes.

### 3.3.2 The Impact of Sampling Delay on Cortisol Awakening Response

Participants who did and did not report a delay of 10 or more minutes between waking up and taking the “waking” sample did not differ in gender distribution, age, or waking time. The proportion of non-compliant participants in the higher and lower subjective social status groups was 70.4 % and 56.3 %; this difference was not significant. The repeated measures analysis comparing the five cortisol samples in participants with and without a sampling delay revealed a significant compliance group by time interaction ( $F_{4,364} = 9.53$ ,  $p < .001$ ). Mean values are shown in Figure 3.1. It is evident that the compliant group showed a rise in cortisol following waking that peaked at 30 minutes, while the non-compliant group did not. The “waking” value was significantly higher in the non-complaint group ( $F_{1,84} = 13.25$ ,  $p < .001$ ), after adjusting for gender, body mass index, smoking and time of waking. Cortisol levels at 20 and 30 minutes post-waking were significantly lower in the non-compliant group ( $F_{1,84} = 5.16$  and 3.90, respectively,  $p < .05$ ). It is likely that cortisol was already declining following the awakening response in the non-compliant group. Including such individuals in any analyses involving psychological or biological factors would be seriously misleading.

### 3.3.3 Social Status and Cortisol Awakening Response

Analysis of the 81 participants without any delays in sampling revealed two significant interactions, between subjective social status and time ( $F_{4,308} = 3.02$ ,  $p = .033$ ), and between gender and time ( $F_{4,308} = 3.17$ ,  $p = .027$ ). There was no three-way interaction, indicating that the associations with social status and gender were independent of one another. These effects are illustrated in Figure 3.2. It can be seen that the higher and lower social status groups did not differ in cortisol level on waking, but that the CAR was greater in the lower status participants. The CAR peaked at 30 minutes in the lower status group, while levels remained steady between 20 and 30 minutes in the higher status group. *Post hoc* analyses indicated that the increase in cortisol between 20 and 30 minutes after waking was significant for the lower status group but that values were unchanged in the higher status group. The socioeconomic difference was confirmed in analysis of the change in cortisol between waking and 30 minutes which was significantly greater in the lower than higher social status group ( $F_{1,74} = 4.44$ ,  $p = .039$ ), after controlling for gender, body mass index, waist to hip ratio, smoking and time of waking. The adjusted increases averaged 11.8 and 6.55 nmol/l in lower and higher social status groups, respectively.

Analyses of objective social status revealed no interaction between time of sample and education level ( $F_{4,308} = 0.19$ ,  $p = .89$ ) or between time and level of financial strain ( $F_{4,308} = 0.77$ ,  $p = .51$ ). Analyses of the change in cortisol between awakening and 30 minutes also revealed no difference between individuals with higher and lower education attainment ( $F_{1,74} = 3.03$ ,  $p = .086$ ), or higher or lower financial strain ( $F_{1,74} = 2.29$ ,  $p = .14$ ) when controlling for gender, body mass index, smoking and time of waking.

### 3.3.4 Social Status, Gender and Cortisol Awakening Response

Figure 3.3 depicts a greater cortisol awakening response in women than men. The mean increase from waking to 30 minutes was 10.8 nmol/l in women and 5.34 nmol/l in men after adjusting for body mass index, waist to hip ratio, smoking, subjective SES status and time of waking ( $F_{1,74} = 4.45$ ,  $p = .038$ ).

### 3.3.5 Factors Associated with Social Status and Cortisol Awakening Response

As noted in Section 3.3.1, higher and lower subjective social status groups differed in educational attainment and financial strain. It was decided therefore to test whether CAR differences were maintained after including these factors as covariates in the analytic model. The number of chronic illnesses and medication count derived from medical notes were also added as covariates. The subjective social status difference in the cortisol increase from waking to 30 minutes remained significant after covarying for age, body mass index, smoking, time of waking, educational qualifications, financial strain, number of chronic illnesses and medication count ( $F_{1,69} = 4.37$ ,  $p = .040$ ). The adjusted increases of 11.9 and 6.08 nmol/l in lower and higher social status groups were similar to those documented without these covariates. These results indicate that the impact of subjective status on the CAR was independent of these cofactors. Similarly, the gender difference in cortisol increases following waking remained significant ( $F_{1,69} = 9.29$ ,  $p = .003$ ), with mean adjusted increases from waking to 30 minutes of 12.8 and 5.16 nmol/l in women and men, respectively.

### 3.4 Discussion

The association between low socioeconomic position and poor health is well-recognized, however, the psychobiological pathways which mediate this relationship are less clear, especially among elderly, non-working populations. The present study hypothesised that higher socioeconomic status individuals would display smaller cortisol awakening response than less privileged groups, and that this might indicate the operation of more health-protective psychobiological processes in this group. The cortisol awakening response can be influenced by non-compliance with sample timing, resulting in inaccurate cortisol profiles. Therefore, it was additionally hypothesised that non-compliant participants would have a higher waking cortisol level and a smaller cortisol awakening response.

#### 3.4.1 Compliance and Salivary Cortisol Sampling

Compliance with the timing of cortisol samples is a problem with studies set in the natural environment. Although participants in the study were given clear instructions to follow about the timing of samples, some 11 % reported a delay of 10 minutes or more in the timing of the first sample after waking. The comparison of compliant and non-compliant participants produced clear and important differences. As predicted, non-compliant individuals showed elevated ‘waking’ cortisol levels and no increase over the 30 minutes after waking. By contrast, the compliant group showed the expected profile of the cortisol awakening response (see Figure 3.1). It is possible that in the non-compliant group, the cortisol awakening response had already peaked by the time of the ‘waking’ sample, so the pattern of cortisol change over the 10 and 20 minute samples reflects the downwards slope of the cortisol awakening response. The findings are in accordance with those described by Kudielka et al (2003), Kunz-Ebrecht et al

(2004a) and Broderick et al (2004) in younger samples. The results also endorse the importance of accurate sample timing. Without this information on compliance, the cortisol awakening response may be misinterpreted as blunted, a profile sometimes associated with poor health (Stone et al, 2001; Kudielka & Kirschbaum, 2003). It is conceivable that subgroups of the population, such as those suffering from fatigue or poor sleep, may have particular problems in the timing of waking. It should be noted that the measure of compliance used in this investigation was based on self-report, and this may not be completely accurate. For example, some participants may have stated that they took samples within a few minutes of waking, when in fact they did not. The use of electronic devices that time saliva samples are beneficial (Kudielka et al, 2003). However, electronic timers also depend on the discrepancy between reported time of awakening and objective time of sampling, so do not completely eliminate self-report problems.

### **3.4.2 Socioeconomic Status and the Cortisol Awakening Response**

With non-compliant participants excluded from the analyses, it was discovered that the cortisol awakening response was greater in lower social status participants, and that this effect was present in both men and women. As predicted, it was also discovered that levels of cortisol on waking did not differ by subjective social status. The results are consistent with those of Kunz-Ebrecht et al (2004a), and suggest that the pattern of elevated cortisol awakening response in lower socioeconomic status groups extends to older individuals beyond retirement age.

Lower socioeconomic status is known to be associated with heightened exposure to adverse psychosocial conditions, including greater financial strain, more acute life events and chronic stress, greater social isolation, and lower sense of control (Marmot et al, 1991; Turner & Marino, 1994; Lachman & Weaver, 1998; Mickelson & Kubansky,

2003). The chronic allostatic load induced by these factors may stimulate dysregulation of biological processes related to disease risk (Seeman et al, 2004). This dysregulation may be indexed by a heightened cortisol awakening response, since positive associations between the cortisol awakening response and chronic stressors such as heavy work demands and general strain have been reported (Schulz et al, 1998; Pruessner et al, 2003; Kunz-Ebrecht et al, 2004b). Although a low cortisol awakening response has been associated with some health problems (Pruessner et al, 1999) (Kudielka & Kirschbaum, 2003; Roberts et al, 2004), it is believe these data support the notion that in old age lower socioeconomic status induces disturbance of neuroendocrine function, accentuating the naturally-occurring increase in cortisol that takes place after waking.

The subjective social status rating was used in this study as the measure of socioeconomic status. The subjective rating was associated with objective indices such as education and financial strain, as has been observed in other studies (Singh-Manoux et al, 2003). However, the lower and higher subjective social status groups differed in the magnitude of the cortisol awakening response even after the objective indicators had been included as covariates. Cortisol awakening response in relation to education and financial strain as objective markers of socioeconomic status was also analysed; but although the cortisol awakening response was somewhat greater in less educated participants and those with greater financial strain, effects were less robust than those observed with the subjective social status rating. These results suggest that in older adults, subjective social status may be particularly useful in providing an aggregate estimate of participants' lifetime social experience that is not captured so effectively by conventional objective markers of socioeconomic status.

### **3.4.3 Timing of Salivary Cortisol Sampling**

It is unclear when exactly post-waking peak cortisol concentrations will occur, and whether this is at the same time for each group of individuals. The timing of cortisol samples was therefore considered carefully in the current design. Results indicate that cortisol concentrations for individuals in the lower social status group peaked 30 minutes post-waking. However, peak cortisol concentration for the higher status group occurred 20 minutes after waking. These results suggest that, if cortisol had been sampled at simply 30 or 45 minutes post-waking, then the group differences observed for social status would not have been found. It is possible that published (and unpublished) null results may be a result of poor timing selection. The findings of the present investigation highlight the importance of early morning cortisol sample timing when assessing between-group differences. This suggests that future studies should attempt to collect saliva samples at regular intervals soon after waking.

### **3.4.4 Depression, Optimism, Gender and the Cortisol Awakening Response**

Depressed mood and clinical depression have been associated with larger cortisol awakening response in a number of studies (Pruessner et al, 2003; Bhagwagar et al, 2003). Depressed mood and low optimism are more prevalent in lower social status groups (Lorant et al, 2003). It is conceivable that the social status difference in cortisol awakening response might reflect differences in depression or optimism. In the present study, no difference in depression and optimism in relation to social status was found. This explanation is therefore unlikely; however, it cannot be ruled out altogether as the comparatively 'young' age of the current sample and the subsequent restricted range of depression scores may indicate that there was too little variability to detect any



significant differences between social status, depression and cortisol awakening response.

### **3.4.5 Gender and the Cortisol Awakening Response**

Another interesting finding was the greater cortisol awakening response in women. This effect was independent of social status and is consistent with previous studies in young and middle-aged adults (Schulz et al, 1998; Wüst et al, 2000; Schlotz et al, 2004). The explanation for the difference in neuroendocrine activation in men and women is not clear. Otte et al (2005) have recently shown in a meta-analysis that cortisol responses to challenge increase with age to a greater extent in women than men. They suggest that a decrease in oestrogen may contribute to the increased cortisol response in women. One might also speculate that women could experience greater strain across all social groups due to additional pressures, such as household chores that are not so prominent in men (Barnett & Hyde, 2001). Similarly, women typically report more worries than men, and this could result in greater stress-related cortisol awakening responses (McCann et al, 1991).

### **3.4.6 Study Limitations**

The study has several limitations that must be considered. It is unlikely that older participants were representative, since this study will have attracted volunteers who were actively interested in issues surrounding health and ageing. The lower life expectancy of lower socioeconomic status men and women inevitably means that those who survive into old age may have a health advantage. This selection bias may have reduced the differences between socioeconomic status groups by involving better functioning individuals. Also, although cortisol response to awakening is relatively stable, measurement over several days would have increased the reliability of these

results. One factor that may have contributed to the pattern of cortisol awakening response is sleep quality. There is evidence that sleep quality is inversely related to socioeconomic status and chronic psychosocial adversity (Steptoe & Marmot, 2003). This may also contribute to the magnitude of the cortisol awakening response (Williams et al, 2005). In the present study, only a simple rating of the previous night's sleep that was unrelated to social status was included. More elaborate measures may identify relevant correlates in the future.

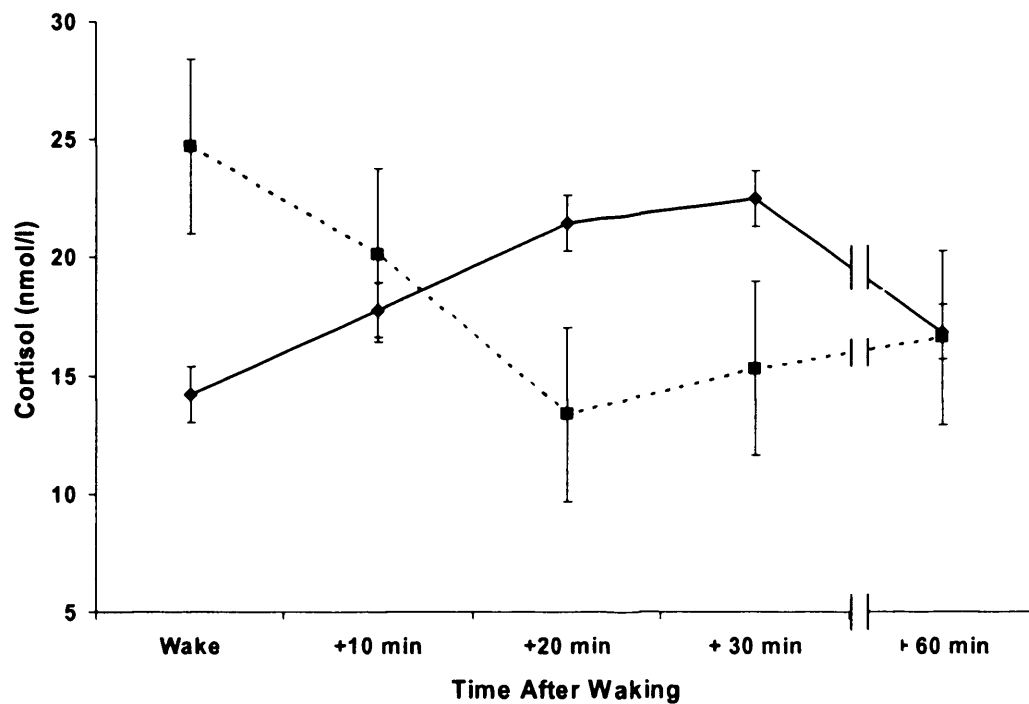
Given that the data in the current study are cross-sectional, it is impossible to determine the causal direction of the association between socioeconomic status, physiological dysregulation and health outcome. One alternative explanation would be that health status may contribute to social and economic position. Although there is some reciprocal influence of socioeconomic status and health (Wadsworth, 1986), the data are more compelling for social causation (Lynch et al, 1997).

### **3.4.7 Conclusion**

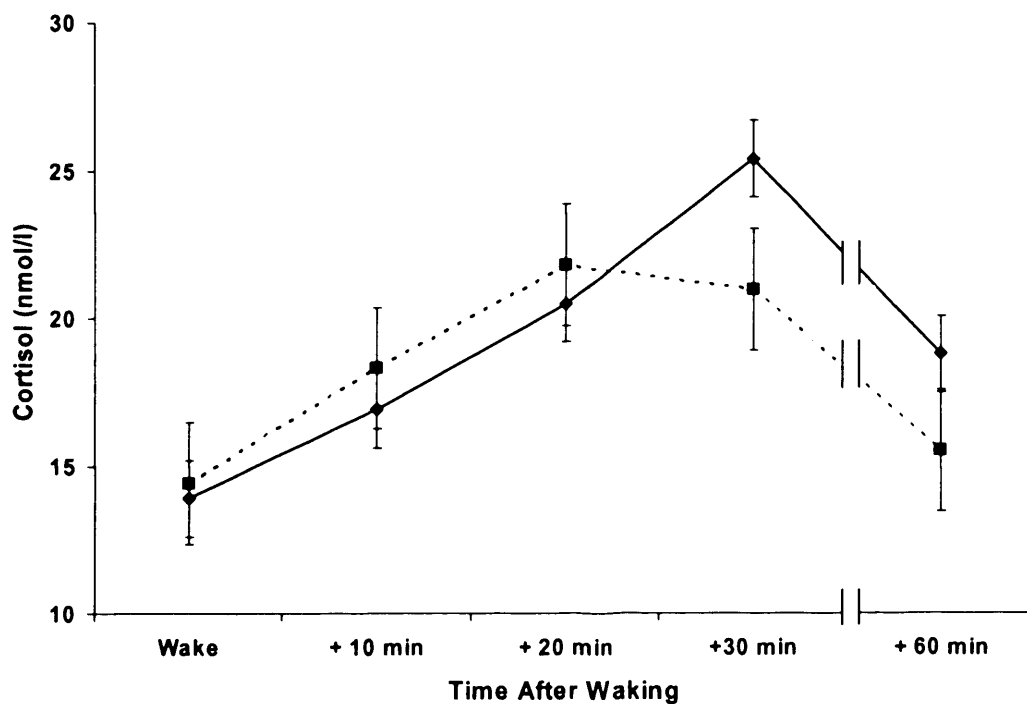
Despite these factors, the findings are consistent with the hypothesis that part of the social gradient in disease is determined by psychobiological pathways stimulated by the neuroendocrine system. More specifically, elderly individuals who see themselves as lower in the social hierarchy of society have a disrupted cortisol awakening response. Interestingly it was also demonstrated that women, regardless of socio-economic position have a greater cortisol awakening response, and that non-compliance with the timing of cortisol sampling can potentially invalidate results related to the cortisol awakening response. A greater understanding of the cortisol awakening response may advance knowledge of the relationship between low socio-economic position and risk of poor health.

**Table 3.1: Characteristics of Higher and Lower Social Status Groups (means  $\pm$  sd)**

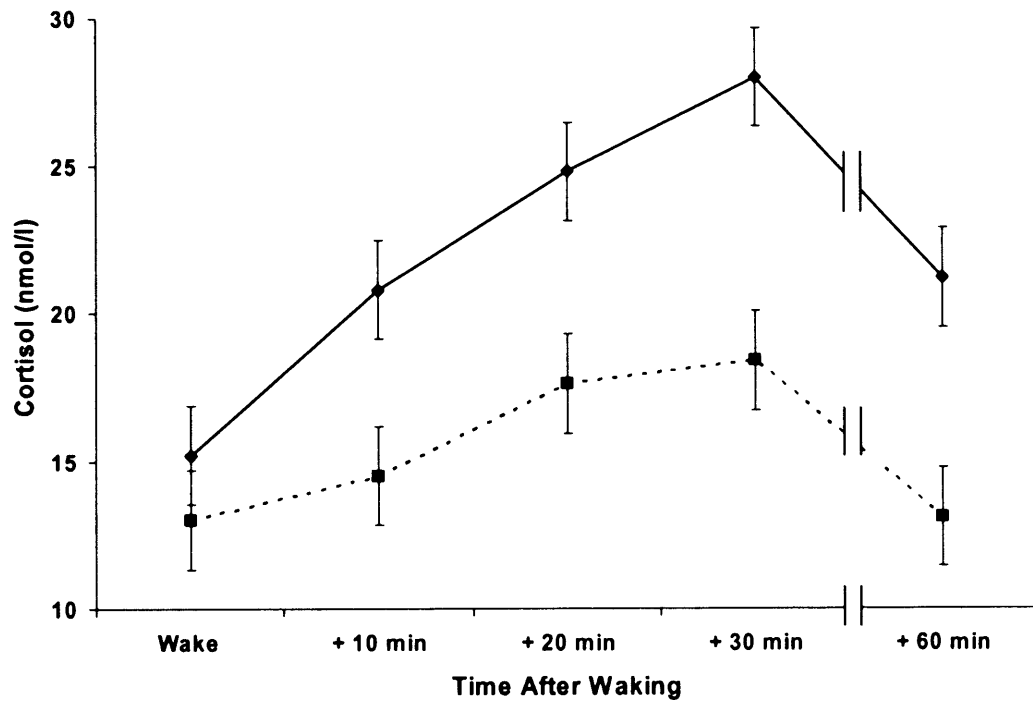
	Higher Status (n = 57)	Lower Status (n = 24)
Men / Women	27 / 30	13 / 11
Age	70.5 $\pm$ 4.2	71.4 $\pm$ 4.0
Educational Attainment: Qualifications	37 (64.9%)	8 (33.3%)
No Qualifications	20 (35.1%)	16 (66.7%)
Age Leaving School (years)	15.9 $\pm$ 1.7	14.7 $\pm$ 1.2
Marital Status: Married	37 (64.9%)	14 (58.3%)
Single/Divorced/Widowed	20 (35.1%)	10 (41.7%)
Chronic Illness (N)	1.18 $\pm$ 1.2	1.58 $\pm$ 1.4
Medications (N)	1.11 $\pm$ 1.1	1.33 $\pm$ 1.2
Body Mass Index (kg/m <sup>2</sup> )	27.8 $\pm$ 4.5	27.0 $\pm$ 4.3
Waist to Hip Ratio	0.904 $\pm$ .09	0.901 $\pm$ .09
Current Smoking or Stopped within 5 years	8 (14.0%)	4 (16.7%)
Walking: None	22 (38.6%)	11 (45.8%)
1-2 / week	18 (31.6%)	3 (12.5%)
>2 / week	17 (29.6%)	10 (41.7%)
Alcohol consumption: Non-drinker	11 (19.3%)	7 (30.4%)
1-5 units / week	21 (36.8%)	9 (39.1%)
> 5 units / week	25 (43.9%)	7 (30.4%)
Financial Strain (1-4)	2.40 $\pm$ 0.75	2.75 $\pm$ 0.66
Optimism (1-5)	3.80 $\pm$ .75	3.53 $\pm$ .64
Depression (0-60)	7.55 $\pm$ 6.29	9.38 $\pm$ 8.24
Sleep Quality (% poor)	16 (28.6%)	5 (21.7%)
Time of Waking	06:54 $\pm$ 57	06:44 $\pm$ 53



**Figure 3.1: Mean Levels of Salivary Cortisol after Waking for Delayed (dashed line) and Non-Delayed (solid line) Samples. Error bars are standard errors of the mean.**



**Figure 3.2: Mean Levels of Salivary Cortisol after Waking for Lower (solid line) and Higher (dashed line) Social Status Groups. Error bars are standard errors of the mean.**



**Figure 3.3: Mean Levels of Salivary Cortisol after Waking for Women (solid line) and Men (dashed line). Error bars are standard errors of the mean.**

## **Chapter 4: Physiological Response to Stress in Young Adults: a Study of Family History of Cardiovascular Disease (Study 3)**

### **4.1 Introduction**

#### **4.1.1 Rationale**

The previous two studies used different methodologies to illustrate that physiological responsivity is related to poor health, and that cumulative strain may be associated with physiological dysregulation (a marker of poor health) across the life course. Both studies suggest that it is the cumulative effect of physiological responsivity / dysregulation which could eventually lead to allostatic load and increased pathology. However, at the moment the link between allostatic load and poor health is speculative, as the cross-sectional nature of the designs did not allow for causal conclusions to be made. This third study therefore adopts a ‘quasi-experimental’ design in order to explore the contribution of allostatic load towards future risk of pathology in an area of psychobiology that is currently under-investigated.

The associations between disturbances in physiological responsivity and physical health, such as cardiovascular disease or hypertension are well established (Step toe & Tavazzi, 1996; Step toe, 1997); however, the role (if any) of hyper-responsivity in the development of these disorders is unclear. As the previous two studies reported here demonstrate, it is often difficult to assess the predictive nature of allostatic load across the life span in participants that are already affected by illness or

impaired health. It is impossible, therefore, to determine whether increased responsivity or changes in response profile are primary or secondary to the illness. An approach that has proved useful for investigating the predictive role of stress responsivity and disease is to compare healthy individuals at high risk of a particular disorder to those with a low risk. This approach allows discovery of whether heightened risk of the disease is associated with disturbances in psychobiological responses prior to the onset of any disorder. One strategy for identifying high and low risk groups is to study people varying in family history of a particular disease. Cardiovascular disease and hypertension have important genetic components, so young people whose parents suffer from these conditions are at elevated risk.

#### **4.1.2 Family History Studies of Hypertension**

The majority of work investigating the contribution of stress reactivity toward cardiovascular disease has focused on the relationship between physiological responsivity and family history of hypertension. Hypertension is classified as abnormally high blood pressure sustained over a period of time. It is associated with an increased risk of heart disease, stroke, kidney failure and other vascular diseases (Ryan, 2002). Recent British Heart Foundation statistics estimate that approximately 4 in every 10 people in the UK have high blood pressure or are being treated for hypertension (British Heart Foundation, 2004). Literature has also shown that in industrialised countries, the risk of becoming hypertensive for an individual with a family history of hypertension is estimated to be up to four times greater than average (Muna, 1993). It is proposed, therefore, that a family history design can determine whether physiological stress reactivity precedes illness onset in individuals most at risk. That is, if disturbed reactivity is present in the offspring of hypertensives and absent in the offspring of normotensives, elevated reactivity can be proposed as a contributing factor to their

increased risk of future hypertension development. Based on this hypothesis, a large and complex literature has addressed whether normotensive individuals with a positive family history of hypertension display greater cardiovascular responses to stress than persons with a negative family history of hypertension.

#### ***4.1.2.1 Cardiovascular Reactivity and Family History of Hypertension***

An early meta-analytic review of family history of hypertension indicated that individuals with a positive family history had greater task reactivity than those with no family risk (Fredrikson & Matthews, 1990). However, later reports suggest that this is not uniformly true for all cardiovascular variables or stress tasks. For example, of the 50 studies, published in the 1970's and 1980's and reviewed by Muldoon et al (1993), a little over one third report family history positive persons to show greater systolic blood pressure reactions to mental stress in comparison to family history negative controls; similarly, diastolic responses were greater among family history positive participants in 32 % of the studies. Among black Americans, blood pressure reactions to the psychological stressors failed to differentiate family history positive and family history negative subjects in nearly all comparisons reported (88 %); while heart rate reactions to laboratory stressors differentiated family history positive and family history negative groups in 37 % of studies. In the 15 years since the review by Muldoon et al a number of other studies investigated the relationship between family history of hypertension and stress reactivity. In an attempt to extend and update the findings of this earlier review, a mini-review of these studies published, in English, over the last 15 years was conducted specifically for this study. A summary of the research can be found in Table 4.1.

All studies were performed on young to middle age normotensive offspring of either hypertensive or normotensive parents. Of the 28 studies included in the table, 25 reported systolic and diastolic reactivity to a stressor, while 26 recorded heart rate



responsivity rates. Of the studies which investigated blood pressure, 36 % found that task-induced diastolic blood pressure was significantly higher in those with a positive family history of hypertension. In addition, 48 % of studies found that systolic blood pressure reactivity was significantly higher among offspring of hypertensive parents; however, the results from three of these studies were only significant for one gender or for one of the tasks employed. With these results discounted, only 36 % of studies investigating systolic blood pressure reported significant results. Similarly, only 27 % of studies discovered that heart rate reactivity differed by parental history of hypertension. It is also interesting that few other cardiovascular variables were investigated; only 6 of the studies examined heart rate variability and / or pre-ejection period. Finally, 5 of the studies (18 %) failed to find any significant differences between cardiovascular reactivity and family history of hypertension.

A possible explanation for these inconclusive findings was investigated in a recent regression-based meta-analysis. Of the 48 papers analysed, Pierce, Grim and King (2005) concluded that cardiovascular reactivity to stress did differ by parental risk, but that patterns in family history differences of cardiovascular reactivity were greatest in situations that elicited the smallest baseline-to-stressor change score for hypertension negative groups, and not a greater reaction to a stressor in the family history positive group as one would expect.

Overall, in the 15 years since the seminal review by Muldoon et al a large body of evidence has been established, however, research has done very little to clarify the true relationship between physiological reactivity and risk of hypertension.

#### ***4.1.2.2 Cardiovascular Recovery and Family History of Hypertension***

In the last 15 years a number of studies have also investigated the importance of cardiovascular recovery from stress in accordance with family history of hypertension.

A majority of this research has demonstrated that delayed cardiovascular recovery may be a marker of hypertension risk, indicating that healthy offspring of hypertensive individuals exhibit a slower and less complete recovery from acute laboratory stressors (Falkner et al, 1979; Anderson et al, 1989; De Visser et al, 1995; Gerin & Pickering, 1995; Hocking Schuler & O'Brien, 1997; O'Brien et al, 1998; Schneider et al, 2003). However, once again this pattern of results does not appear to be uniform, as other studies have found that family history positive and family history negative individuals recover at comparable rates (Lawler et al, 1998).

#### ***4.1.2.3 Family History of Hypertension: Methodology Review***

Although many studies report a significant effect of parental history on cardiovascular reactivity, the literature is complicated by the large number of negative findings and inconsistency regarding the testing conditions under which family history differences in reactivity are most prominent. These inconsistencies have led several authors to question the role of exaggerated reactivity as a causal factor in the development of hypertension (Pickering & Gerin, 1990; Rosenman & Hjemdahl, 1991). Others argue that sufficient evidence of family history differences in cardiovascular reactivity exists to warrant further clarification of its place in the causal chain of events leading to hypertension (Schneiderman et al, 2000). Those arguing in support of a relationship between family history and elevated reactivity to stress cite methodological differences between studies as factors contributing to inconsistencies in the literature. Among the issues mentioned in several reviews are stressor type, unclear methodology, demographic variation and a failure to confirm parental hypertension status (Lovallo & Wilson, 1992a; Matthews & Rakaczky, 1986; Fredrikson & Matthews, 1990; Pickering & Gerin, 1992; Turner, 1994).

The first possible source of inconsistency is the choice of stressor. There may be qualitative differences in the cardiovascular responses elicited by different tasks which interact with family history of hypertension to produce group differences in reaction to some, but not all, stressors. It can be seen in Table 4.1 that the cold-pressor task elicited a higher proportion of negative results than active coping challenges. Similarly, a review of task recovery and family history studies reported that the relationship between cardiovascular recovery and hypertension status was typically associated with the use of ‘active laboratory stressors’ (Hocking Schuler & O’Brien, 1998). Furthermore, certain laboratory stressors, such as the cold-pressor test may elicit responses which are not representative of those produced by day-to-day stressors. There is a need therefore, for studies to investigate family history and cardiovascular reactivity to more naturalistic stressors; for example, the study by Adler et al (1994b) which discovered that participants with a positive family history of hypertension had significantly higher blood pressure responses to blood donation.

The second possible source of inconsistency in family history research may derive from methodological confusions. As numerous reviews and Table 4.1 illustrate, studies differ widely in sample size, with the majority being small and composed solely of men. Other problems include lack of uniformity in consideration of methodological issues. There are many variables which could potentially affect cardiovascular responsivity or family history status and these are not considered consistently across studies. For instance, there are a number of individual difference variables (e.g. gender, ethnicity, fitness level, smoking status, weight) and methodological variables (e.g. length of recovery period, calculation of reactivity / recovery scores) which should be consistent or credible when conducting research of this kind (Hocking Schuler & O’Brien, 1998).

Another possible explanation for variability in results is the failure to confirm parental hypertension status. This was true for 19 % of the studies reviewed in Table 4.1 where hypertension status of parents relied on participants own self-report. In addition, the common practice comparing offspring with one or more hypertensive parents with participants whose parents are normotensive may not be the most powerful design, although this method was employed by 79 % of the studies reviewed in Table 4.1.

Another concern is the negative results reported in several well-designed studies (i.e. Perini et al, 1988; Ravogli et al, 1990; Perini et al, 1990; Gerin & Pickering, 1995). This led some authors to investigate possible third factors which may account for the differences between family history groups found in certain studies. It has recently been shown that family history positive participants who score highly on avoidant coping have greater task reactivity than family history negative participants and family history positive participants with low avoidant coping (Schwerdtfeger et al, 2005). Similarly, it was found that behavioural responses to interpersonal tasks could mediate the relationship between family history and cardiovascular reactivity (Semenchuk & Larkin, 1993). For example, Vögele and Steptoe (1993) reported that high risk boys (defined as participants with one or more parent with a history of coronary heart disease, hypertension, or a resting blood pressure  $\geq 140/85$  mmHg), who also reported high levels of anger inhibition had greater systolic blood pressure responses to two stressful tasks. Crucially, high anger inhibition was not more frequent among high family risk subjects; it was therefore the combination of family history and anger expression which was important. These results also support the authors' previous findings which showed that young men with high 'normal' blood pressure who had a tendency toward neurotic inhibition (high anxiety accompanied by anger inhibition and self-concealment) were

more responsive to mental stress tasks than subjects without these characteristics (Vögele & Steptoe, 1992). It is suggested, therefore, that behavioural responses, such as the tendency to express anger may interact with familial factors in determining responsivity patterns that may be indicative of raised risk of future cardiovascular disease.

In summary, the study of family history and hypertension may be an important way to determine whether cardiovascular responsivity is a contributory factor in hypertension progression; although, a review of methodological components is needed before the family history design is truly valuable. A vast amount of work has been carried out on cardiovascular reactivity and hypertension and there is now scope to adapt this theory and methodology to investigate other areas of health using a family history design. One context in which the family history model could be used to investigate the relationship between stress responsivity and disease development is cardiovascular disease.

### **4.1.3 Family History Studies and Cardiovascular Disease**

Cardiovascular disease is one of the leading causes of death and morbidity in western societies. It encompasses dysfunctional conditions of the heart, arteries and veins, and includes disorders such as arteriosclerosis, angina, myocardial infarction and stroke. There are numerous risk factors for cardiovascular disease but the most recognised include raised blood pressure, diabetes mellitus, smoking, high cholesterol and obesity (Rashid et al, 2003; Briganti et al, 2005). Due to the severity and prevalence of the disease, examining the risk factors surrounding cardiovascular disease has become essential.

#### **4.1.3.1 Cardiovascular Responsivity and Cardiovascular Disease**

Excessive cardiovascular responsivity to stress has been implicated in the development of cardiovascular disease, and heightened cardiovascular responsivity has been associated with coronary atherogenesis, increased cardiac mass, plaque rupture, thrombus formation, vasospasm and arrhythmogenesis (Manuck, 1994); however, because these studies are often carried out with unhealthy samples, it is not possible to determine whether stress reactivity precedes the onset of disease. Nevertheless, there is still theoretical evidence which suggests that increased cardiovascular responsivity could lead to cardiovascular disease.

Atherosclerosis is a disease of the large arteries in which thickening of the arterial intima results from intracellular and extracellular accumulation of plasma lipids, proliferation of smooth muscle cells and migration of macrophages into the intima. Thought to be initiated by injury to the arterial endothelium, atherosclerotic lesions progress through stages of fatty streaking, the development of fibrous plaques, leading to possible plaque rupture and thrombus formation. Marked stress-induced cardiovascular reactions are often responsible for flow disturbances which promote these early atherosclerotic events and endothelial injury (Ross, 1999). One of the principal models relating cardiovascular reactivity to cardiovascular disease is individual differences in response. In accordance with the theory of allostatic load, it is this assumption which suggests that repeated cardiovascular hyperresponsivity will result in cumulative detrimental alterations in cardiovascular structure and function in some individuals.

Research supporting the theory of allostatic load suggests that heightened responsivity to stress can eventually lead to cardiovascular disease. For example, men with higher systolic and diastolic blood pressure during a laboratory task have been

shown to be hypertensive at 15 year follow-up (Light et al, 1992); while three longitudinal studies with follow-up periods ranging from 5 to 10 years reported that elevated cardiovascular recovery is also associated with future development of hypertension (Borghi et al, 1986; Tanji et al, 1989; Singh et al, 1999). However, it is not always possible to conduct longitudinal studies. It is known that a positive family history of cardiovascular disease is strongly associated with future disease incidence, even when environmental risk factors are accounted for (Williams et al, 2001b; Andresdottir et al, 2003). It may therefore be possible to determine whether physiological dysfunction precedes cardiovascular disease by employing a family history design. To date, few studies have investigated cardiovascular reactivity and family history of cardiovascular disease generally, as much of the research, as demonstrated earlier, focused on family history of hypertension. A few studies have found that normotensives with positive family histories of coronary artery disease manifested by myocardial infarction, exhibited greater blood pressure increases or less attenuation of total peripheral resistance than those with a negative family history, to a variety of stressors (Stoney et al, 1988; Treiber et al, 1991; Treiber et al, 1993). In spite of this, no studies to date have examined the relationship between cardiovascular responsivity and family history of cardiovascular disease in terms of other risk factors such as obesity, hypertension, high cholesterol and diagnosed heart disease or diabetes.

#### ***4.1.3.2 Cortisol Responsivity and Cardiovascular Disease***

A large amount of research has focused on the impact of cardiovascular responsivity and cardiovascular disease; however, it may be possible that the effects of increased glucocorticoids could also be harmful to the vasculature. It is common for patients with Cushing disease to experience early death from stroke (Colao et al, 1999) and pre-Cushing syndrome patients often present arterial hypertension, lipid

abnormalities, impaired glucose tolerance, type II diabetes mellitus, and abnormal haemostatic parameters (Tauchmanova et al, 2002). In addition, cortisol has been found to induce atherosclerosis in animal models (Stambler et al, 1954), while observational studies in humans have discovered that corticosteroid-treated rheumatoid arthritis and lupus patients have significantly more atherosclerosis than those not treated with steroids. Risk of atherosclerosis is also related to the cumulative dose of corticosteroids (Manger et al, 2003; Vlachoyiannopoulos et al, 2003). Glucocorticoids have been found to influence body composition, plasma lipoprotein metabolism, endothelial function, oxidative stress and vascular tone all of which are precursors for atherosclerosis and cardiovascular disease (Girod & Brotman, 2004). There appears to be a clear association between cortisol increase and cardiovascular disease but whether this neuroendocrine alteration is primary or secondary to disease onset remains to be investigated. To date just one study has examined cortisol reactivity and family history of hypertension. Fredrickson et al (1991) discovered that healthy participants with a family history of hypertension had elevated cortisol increases to stress, but only for active coping (not physical) tasks. No relationship was found between cortisol and family history of myocardial infarction. There is now a need to examine the relationship between cortisol reactivity and family history of cardiovascular disease risk factors in greater detail.

#### ***4.1.3.3 Cytokine Responsivity and Cardiovascular Disease***

The immune system is highly sensitive to acute psychological stress, and acute stress-induced increases in proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF $\alpha$ ) have been reported (Dobbin et al, 1991; Maes et al, 1995; Maes et al, 1998; Lutgendorf et al, 1999; Steptoe et al, 2001; Owen & Steptoe, 2003; Brydon et al, 2004), although not consistently



(Ackerman et al, 1998; Dugue et al, 2001). Atherosclerosis is also, in part, an inflammatory disease of the subendothelium (Ross, 1999), and pro-inflammatory cytokines are suggested to be associated with hypertension, obesity and diabetes (Libby et al, 2002; Fahdi et al, 2003). For example, elevated levels of circulating IL-6 have been shown to predict future myocardial infarction in healthy men and women (Ridker et al, 2000c; Ridker et al, 2000a), and mortality in an older cohort (Harris et al, 1999). The plasma concentration of  $\text{TNF}\alpha$  is also raised in cardiac patients who experience recurrent coronary events (Ridker et al, 2000b), while outcomes in patients with unstable angina are poor among individuals with elevated IL-1Ra (interleukin-1 receptor antagonist) and IL-6 levels (Biasucci et al, 1999; Lindmark et al, 2001). The observation that cytokines are responsive to mental stress suggests that they may be involved in the pathways through which psychosocial factors influence cardiovascular disease risk.

It is possible, therefore, that in accordance with the theory of allostatic load, increased cytokine reactivity could, over time lead to the development of cardiovascular disease or its risk factors. However, the predictive value of stress-induced cytokine activation and cardiovascular disease is at present only speculative. The use of family history design would be useful in examining whether stress-related cytokine activation precedes cardiovascular disorder in those most at risk.

#### **4.1.4 Family History Studies and Adiposity**

One of the major risk factors for cardiovascular disease is obesity, which is now the second leading cause of preventable death in the United States (Melanson et al, 2001). In general, the prevalence rates of overweight and obesity have increased three-fold since 1980 (Eisenmann, 2003), with 21 % of British women and 17 % of British men now considered obese (National Audit Office, 2001). Obesity also renders both

sexes more vulnerable to diabetes (Kelley, 1998), cardiovascular diseases (McGinnis & Foege, 1993), hypertension (Eckel, 1997; Heyka, 1998) and impaired glucose tolerance (Ljung et al, 2000). An estimated 40-60 % of cases of hypertension are associated with obesity, while 85-90 % of all patients with diabetes mellitus are obese (Melanson et al, 2001). However, contrary to expectation, a recent review of seven large-scale epidemiological health surveys reported that the secular increase in obesity could not be linked to available self-report data on changes in physical activity or diet (Eisenmann, 2003). Although the author advises that measurement issues must be considered, it is also recommend that other factors associating energy intake and expenditure in relation obesity and cardiovascular disease be investigated. It is suggested that research should now investigate individual differences in energy expenditure and metabolism in relation to fat storage and use. One mechanism which could be responsible for the development of obesity, or explain the link between adiposity and cardiovascular disease is the role of individual physiological response to stress.

#### ***4.1.4.1 Cortisol Responsivity and Adiposity***

The majority of research exploring the relationship between physiological responsivity, adiposity and cardiovascular disease has investigated HPA axis dysregulation. Numerous studies suggesting a link between cortisol reactivity and adiposity found that obesity is associated with increased cortisol clearance, higher than average cortisol turn over, and altered cortisol metabolism in adipose tissue (Rebuffe-Scrive et al, 1990; Bujalska et al, 1997; Vicennati & Pasquali, 2000). Associations between cortisol and adiposity have also been seen in people with Cushing's disease. However, it is less clear whether a relationship between stress-induced cortisol and fat distribution among healthy individuals with normal basal neuroendocrine parameters exists. In support of this theory, Epel et al (2000) discovered that women with a high

waist to hip ratios secreted significantly more cortisol during the first stress session than women with low waist to hip ratio. Furthermore, women with high waist to hip ratio lacked habituation to a familiar stressor. Marin et al (1992) and Rosmond et al (1998) also reported a positive relationship between stress-induced cortisol production and central adiposity.

There are several widely held explanations for the association between cortisol reactivity and adiposity. The first suggests the involvement of the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1). 11 $\beta$ -HSD2 converts cortisol to its inactive form cortisone in the liver, while 11 $\beta$ -HSD1 reactivates cortisone to active cortisol. It has been discovered in obese individuals that reactivation of cortisone to cortisol by 11 $\beta$ -HSD1 is impaired so that plasma cortisol levels tend to fall. There may then be a compensatory increase in cortisol secretion, which is often associated with weight gain (Andrew et al, 1998; Stewart et al, 1999; Rask et al, 2001). A second explanation for the association between adiposity and neuroendocrine reactivity involves the peptide hormone leptin. Produced by adipocytes, leptin has inhibitory actions on the HPA axis and appears to be an afferent signal that indicates the amount of fat storage (Nye et al, 2000). Consequently, leptin and cortisol display an inverse circadian rhythm and plasma leptin concentrations correlate closely with adipose tissue mass in humans (Licinio et al, 1997; Fried et al, 2000). Under normal circumstances regular HPA function ensures leptin signals satiety; leptin is therefore partly responsible for weight stabilisation. However, over time, repeated cortisol release in response to stress may cause an excess release of leptin leading to leptin resistance, where the body is unable to accurately assess satiation levels. As a result energy intake and expenditure may become unbalanced causing weight gain to ensue (Leal-Cerro et al, 2001).

Despite evidence of an association between cortisol reactivity and adiposity in healthy individuals, these studies still fail to elucidate whether a neuroendocrine dysregulation precedes or follows weight gain. It is unclear therefore, whether repeated cortisol reactivity contributes to weight gain over time, or alternatively whether cortisol dysregulation due to weight gain leads to cardiovascular disease. Body weight is partly heritable, so a family history design could be used to explore this relationship further.

#### ***4.1.4.2 Cardiovascular Responsivity and Adiposity***

Although the majority of research has focused on endocrine responsivity and adiposity, a few studies have examined the relationship between fat distribution and cardiovascular response to acute psychological stress. In a sample of young white men, Jern et al (1992) reported that waist to hip ratio was correlated with total peripheral resistance during mental stress. In a subsequent study, Barnes et al (1998) found that those in the upper tertile of waist to hip ratio showed greater peak blood pressure increases in response to stress than those in the low tertile. Furthermore, Waldstein et al (1999) discovered that central adiposity was associated with blood pressure and heart rate responses to cognitive stress in older men and women. Waist to hip ratio has also been associated with BP reactivity in school teachers (Steptoe & Cropley, 2000), and reduced heart rate variability in obese children (Nagai et al, 2003). In addition, Davis et al (1999) discovered that blood pressure, total peripheral resistance and cardiac output reactivity were associated with central adiposity in women. These findings are important as they suggest that individuals with central adiposity have cardiovascular disturbances in response to stress. However, because the studies were all conducted on participants with high pre-existing adiposity levels it is impossible to determine whether cardiovascular reactivity to stress is a contributory factor in weight gain, or a mediating factor between obesity and cardiovascular disease which follows increased adiposity.

This could then explain the relationship between obesity and high incidence of cardiovascular disease (as mentioned in Section 4.1.3). A recent study by Steptoe and Wardle (2005) reported that disturbances in cardiovascular responsivity, manifest through impaired post-stress blood pressure and cardiac index recovery, were associated cross-sectionally with body mass index and waist to hip ratio. More importantly, after a three year follow-up period waist to hip ratio was predicted by impaired post-task systolic blood pressure and cardiac index in men, independent of baseline adiposity and cardiovascular levels, age, socioeconomic status, smoking and alcohol consumption. In summary, the authors speculate that stress responsivity is predictive of abdominal weight gain over time. This third study will, therefore, employ a family history design to determine whether this relationship persists in young healthy adults also.

#### ***4.1.4.3 Cytokine Responsivity and Adiposity***

Recent data suggests a direct association between obesity and inflammation. Cytokines, in particular IL-6, have been reported to be powerful stimulators of the HPA axis. It may be the case, in accordance with the theory of allostatic load, that stress-induced cytokine and subsequent cortisol production could over time lead to an increase in adiposity in more reactive individuals. However, it has also been found that cytokines such as IL-6, TNF $\alpha$  and IL-1 are produced by adipocytes and may increase in the context of obesity (Mohamed-Ali et al, 1997; Yudkin et al, 1999). It can be conversely hypothesised that the relationship between cytokine increase and adiposity could explain the association between adiposity and cardiovascular disease rather than acting as contributing factor towards weight gain. To date, studies have not explored the relationship between cytokine reactivity and adiposity. Work is needed therefore to elucidate the role of stress-induced cytokines in obesity and cardiovascular disease.

#### ***4.1.4.4 Summary of Physiological Responsivity, Cardiovascular Disease and Adiposity***

The literature presented in this review shows a strong association between physiological responsivity and cardiovascular disease. Based on the theory of allostatic load it is feasible to suggest that heightened stress responsivity may contribute to future disease development. It is believed therefore, that individuals with heightened reactivity to, or prolonged recovery from a stressor, if experienced frequently, could over time cause advanced wear and tear on the body's cardiovascular system resulting in an increased vulnerability to diabetes mellitus, cholesterol, hypertension, stroke or atherosclerosis (Björntorp & Rosmond, 2000). The use of a family history design, first investigating susceptibility to hypertension and then more globally cardiovascular disease, will help to determine whether cardiovascular, neuroendocrine and cytokine responsivity precedes disease onset.

Based on the evidence presented, it is also known that obesity is a major risk factor for cardiovascular disease independent of cholesterol, blood pressure, and diabetes. In addition, a strong association between physiological responsivity and visceral adiposity is reported. Although the direction of this relationship is unclear, some authors do tentatively conclude that elevated visceral fat mass is a direct consequence of long term physiological stress responsivity, and that this individual vulnerability is based on genetic susceptibility (Björntorp, 2001). It is known also that a substantial number of children with obese parents will become obese in adulthood (Whitaker et al, 1997). A family history design using parental adiposity levels may therefore prove valuable when examining whether stress responsivity occurs before increased adiposity in those most at risk of weight gain. That is, because adiposity distribution is partly heritable it may be possible to predict that young normotensives

with heightened physiological responsivity, whose parents are overweight, are at greater risk of future obesity than those participants whose parents fit within normal weight ranges. However, because the relationship between adiposity and stress-responsivity to date is correlational, there is the possibility that physiological responsivity may not be a predictive factor in the relationship between obesity and cardiovascular disease. It may be that the relationship between adiposity and responsivity is due to obesity-induced cardiovascular, neuroendocrine and cytokine disturbances which then (as proposed earlier) go on to increase vulnerability to cardiovascular disease. With the use of a family history design, the present study will attempt to separately explore whether physiological responsivity precedes hypertension, cardiovascular disease and adiposity development in those most at risk of these three disorders.

#### ***4.1.4.5 Methodological Critique***

In order to assess the relationship between physiological responsivity, cardiovascular disease and its risk factors certain methodological improvements are necessary. For example, many of the studies reviewed used small samples of male, white subjects, although the data from some studies suggests that hypertension and cardiovascular responsivity may differ by gender (Ballard et al, 1993; De Visser et al, 1995; Al' Absi et al, 1999) and race (Johnson et al, 1991; Mills et al, 1993). More studies need to target and explore the differences between males and females and individuals from different ethnic backgrounds. In addition, a number of studies investigated the effects of stress responsivity on older samples or those already afflicted by the disorder of interest. To determine the direction of events between physiological responsivity and cardiovascular disease, research needs to employ young, healthy participants who, as yet do not suffer from any cardiovascular disease-related symptoms.

Evidence also concludes that recovery is over-looked in stress physiology research (Linden et al, 1997). A meta-analysis of 69 studies investigating recovery and hypertension risk factors reported that the average recovery period used was only 13.4 minutes, while 32 % of these studies assessed a recovery period of 4 minutes or less (Hocking Schuler & O'Brien, 1997). The use of single, short recovery periods means that individual variability in recovery time cannot be properly evaluated. Regarding the calculation of recovery success, Hocking Schuler and O'Brien (1997) found that 38 % of studies did not account for baseline or stressor levels when reporting recovery values. Instead raw values were used to conduct between-group comparisons. Use of raw values may be problematic because it is difficult to determine whether observed between-group differences were a result of differences already present at baseline, differences in reactivity to the stressor, or differences in recovery. The authors therefore recommend the use of multiple recovery assessments over a longer time period, taking into account baseline or reactivity levels.

The last methodological issue which needs consideration concerns the classification of family history risk. A positive family history is usually interpreted as at least one parent with a particular disorder; while the more powerful comparison of strong versus absent family history (e.g. biparental hypertension versus biparental normotension), or family history from more than one generation (parents and grandparents) is employed in only a few studies. A more robust classification of family history is needed when studying the relationship between stress responsivity and risk of cardiovascular disease.

#### **4.1.5 Investigation Aims**

Taking into account these methodological concerns, this third study was designed to investigate whether disturbances of physiological responsivity could be



determined in people with a raised risk of cardiovascular disease. A useful approach for this type of investigation is to employ a family history design. Using a large sample of male and female healthy normotensive volunteers, the present study aimed, primarily, to investigate whether cardiovascular, neuroendocrine and cytokine reactivity to, and recovery from, two mental stress tasks differed according to family history risk of cardiovascular disease (defined by familial levels of cholesterol, hypertension, heart disease and diabetes). Methodological issues mentioned in Section 4.1.4.5 were considered and analyses controlled for various factors including the participant's own baseline physiological levels, body mass index, waist to hip ratio and smoking status. Physiological responses assessed included systolic blood pressure, diastolic blood pressure, heart rate, heart rate variability, pre-ejection period (cardiovascular), cortisol (neuroendocrine) and IL-6 (cytokine) variables. The study also proposed to use strict methodology to replicate and extend previous research which examined the relationship between cardiovascular responsivity and risk of hypertension. The relationship between neuroendocrine and cytokine stress responsivity was also examined in accordance with family history risk of hypertension, while the association between neuroendocrine, cytokine, cardiovascular responsivity and students' own adiposity (measured by waist to hip ratio and body mass index) was explored. Finally, the relationship between cardiovascular, neuroendocrine and cytokine responsivity and family history of adiposity was assessed as a separate risk factor for cardiovascular disease. In doing so, the study attempted to explore whether physiological responsivity could be predictive of future obesity development, or whether physiological responsivity is a component both of adiposity and cardiovascular disease.

#### 4.1.6 Hypotheses

It was hypothesised, that

- Participants with a positive family history of hypertension, compared with participants with no family history of hypertension, would demonstrate significantly greater cardiovascular, neuroendocrine or cytokine responsivity to two laboratory stressors.
- Participants with a positive family history of cardiovascular disease (including high cholesterol, coronary heart disease, diabetes or hypertension) would demonstrate significantly larger cardiovascular, neuroendocrine and cytokine responses to two stressors in comparison to participants with no family history of cardiovascular disease.
- Participants with high adiposity (defined by waist to hip ratio and body mass index) would demonstrate a greater cardiovascular, neuroendocrine and cytokine response to stress than participants with low adiposity.
- Participants with a family history of high adiposity, compared to participants with a family history of low adiposity, would demonstrate greater cardiovascular, neuroendocrine or cytokine responsivity to the two laboratory tasks.

It is recognised that each of the hypotheses are not independent of each other and that there are strong correlations between cardiovascular disease, hypertension and adiposity. However, for exploratory reasons these factors will be considered separately. The problems associated with this are discussed later in the chapter.

Table 4.1: Summary of Studies Examining Physiological Reactivity and Family History of Hypertension (1990-2005)

Author(s)	Sample Size	Family History Classification	Task	Null Results	Significant Results
Adler et al (1994)	469	None versus one hypertensive parent	Anticipation of blood donation	HR	SBP, DBP
Adler & Ditto (1998)	24 male undergraduates	None versus one hypertensive parent	4 emotional event interviews	DBP, HR	SBP
(Adler & Ditto, 1998)d}					
Al' Absi et al (1999)	128 (21-40 yrs)	None versus one hypertensive parent (subject self-report)	Cold-pressor	HR, SBP, rate pressure product	DBP (men only)
Al' Absi et al (2000)	54 females	None versus one hypertensive parent (subject self report)	Cold-pressor	SBP, DBP, HR	
(Al' Absi et al, 2000)d}					
Ballard et al (1993)	49 children mean age 12.5 yrs	None versus one hypertensive parent	Verbal interactions Mirror tracing task Digit span task	DBP HR	SBP (male only)
De Visser et al (1995)	99 young adults	Two normotensive versus two hypertensive parents (clinical assessment)	Memory task, Reaction time test	DBP, HR, PEP, LVET, HRV	SBP (mem), TPR (rt)
Everson et al (1992)	105 male undergraduates	Low, moderate, high risk based on parental hypertension and own SBP	Mental arithmetic, Cold-pressor	SBP, DBP, rate pressure product (cold-pressor), HR both tasks	SBP, DBP, rate pressure product
(Everson et al, 1996)d}					
France & Stewart (1995) (France & Stewart, 1995)d}	80 young male adults	None versus one hypertensive parent	Cold-pressor	MAP, HR	
Frazer et al (2002)	64 undergraduates (18-46 yrs)	None versus one hypertensive parent	Mental arithmetic, mirror tracing, interpersonal role-plays	DBP, HR	SBP
(Frazer et al, 2002)d}					
Fredrikson et al (1991)	60 middle aged adults	None versus one hypertensive parent (subject self report)	Mirror tracing, mental arithmetic, Stroop task, cold-pressor, isometric handgrip	Adrenaline, noradrenaline, cortisol, HR (to physical stressors)	Adrenaline, noradrenaline, cortisol, HR

Author(s)	Sample Size	Family History Classification	Task	Null Results	Significant Results
Gerin & Pickering (1995)	537 undergraduates	None versus one hypertensive parent	Mental arithmetic	SBP, DBP, HR	
Johnson et al (1991)	38 black males	None versus one hypertensive parent	Mental arithmetic, cold-pressor	DBP, HR, forearm blood flow, forearm vascular resistance	SBP
Manuck et al (1996)	90 college students (18-25 yrs)	One, two or no hypertensive parents	Stroop task, mental arithmetic, mirror tracing	SBP, DBP, HR, PEP, LVET	
Lamensdorf & Linden (1992) (Lamensdorf & Linden, 1992)	64 undergraduates	None versus one hypertensive parent	High distress speech task, mental arithmetic	SBP, HR	DBP
Lawler et al (1998)	203 college students	None versus one hypertensive parent (subject self report)	Mental arithmetic, anger recall interview		SBP, DBP, HR
Marrero et al (1997) (Marrero et al, 1997)	33 males, 21-35 yrs	None versus one hypertensive parent (subject self report)	Reaction time, mental arithmetic	HR, SV, CO	SBP, DBP, vascular resistance
Maver et al (2004)(Maver et al, 2004)	105 medical students	None versus one hypertensive parent <55yrs (self report)	Cold-pressor	SBP, DBP, HR	HRV
Miller & Ditto (1991)(Miller & Ditto, 1991)	48 young males	None versus one hypertensive parent	Shock avoidance video game	SBP, DBP	HR, blood volume pulse, forearm blood flow
Miller (1992)	48 young males	None versus one hypertensive parent (detailed parent and doctor interview)	Shock avoidance video game	SBP, DBP	HR, forearm blood flow, forearm vascular resistance
Miller (1994)(Miller, 1994)	24 young males	None versus one hypertensive parent (subject self report)	Cold-pressor, Isometric hand grip Film, Shock avoidance video game	HRV	HR (hand grip and video game only)

Author(s)	Sample Size	Family History Classification	Task	Null Results	Significant Results
Miller & Sita (1994)(Miller & Sita, 1994)	44 women undergraduates	None versus one hypertensive parent	Shock avoidance video game, Speech task, Cold-pressor	HR, SBP, CO, SV, PEP, TPR	DBP (speech task)
Miller et al (1995)(Miller et al, 1995)	40 male undergraduates	None versus one hypertensive parent (subject self report)	Shock avoidance video game and sodium loading	SBP, DBP, forearm blood flow, forearm vascular resistance	TPR, nor adrenaline, SV, CO
Pierce & Elias (1993)(Pierce & Elias, 1993)	College students	None versus one hypertensive parent	Two tests of cognitive function	SBP, DBP	HR
Sausen et al (1991)	99 male undergraduates	None versus one hypertensive parent (detailed parent interview)	Mental arithmetic, Cold-pressor	HR	SBP, DBP, Pressure product response
Schneider et al (2003)	42 (20-35 yrs)	None versus one hypertensive parent (clinical assessment)	Mental arithmetic	SBP, DBP, MAP, CO, SV, TPR	Cardiac index, PEP
Semenchuk & Larkin (1993)	40 male undergraduates	None versus one hypertensive parent	Interpersonal speech tasks	HR DBP	SBP
Treiber et al (1993)	195 boys (11.5 mean age)	No hypertensive parent or grandparent versus one hypertensive parent and grandparent	Video game, Cold-pressor	SV	Systemic vascular resistance, SBP, DBP, cardiac index, HR
Vögele and Steptoe (1993)	60 boys (12-16 yrs)	None versus one hypertensive parent (clinical assessment)	Mental arithmetic, mirror tracing	SBP, DBP, HR	

Table Abbreviations: CO = cardiac output; DBP = diastolic blood pressure; HR = heart rate; HRV = heart rate variability; LVET = left-ventricular ejection time; MAP = mean arterial pressure; PEP = pre-ejection period; RPP = rate pressure product; SBP = systolic blood pressure; SV = stroke volume; TPR = total peripheral resistance.

## **4.2 Method**

### **4.2.1 Participants**

The study involved 103 male and female students aged between 18 and 25 years. Participants were screened by structured interview to ensure that they were generally fit and healthy, not taking any medication (including antidepressants), without cold or flu symptoms and had at least one contactable blood-related parent (see Appendix XVII and XVIII for participant instruction sheet and study protocol). Participants were told to avoid taking aspirin, ibuprofen or antibiotics for 10 days prior to the session, and caffeine and alcohol 12 hours before the session. Of the 103 participants 34 were male (33 %) and 69 were female (67 %). The majority of participants were university students. Informed consent was gained from each participant and ethical approval was granted by University College Hospital Medical Research Ethics Committee (Appendix III).

### **4.2.2 Laboratory Mental Stress Tasks**

Two mental stress tasks were administered in a fixed order and completed by participants in a standardised laboratory setting. The tasks were each administered for a 5 min period and were designed to induce moderate physiological activation. The test period included a public speaking and cognitive task. This combination has been found to elicit perceptions of social-evaluative threat and uncontrollability which lead to measurable increases in cortisol (Dickerson & Kemeny, 2004).

#### **4.2.2.1 Cognitive Stroop Task**

A modified computerised version of the Stroop colour-word interference task was administered for 5 minutes (Appendix XIX). During this task participants were

presented with a target word in the centre of the screen which was printed in an incongruent colour (green, red, blue, yellow). The participant was then asked to match the colour that the target word was printed in, with the correct word from four options at the bottom of the screen. The task ran at varying speeds, increasing when a participant performed well in order to keep pressure constant throughout the 5 minute testing period. Participants were given a 30 second practice period and were instructed that accuracy and speed of response would be recorded. The Stroop task has induced moderate stress responses in studies examining cardiovascular reactivity (Steptoe et al, 1999; Steptoe et al, 2003a) and changes in the immune and endocrine system (Steptoe et al, 2002; Brydon et al, 2004).

#### **4.2.2.2 Public Speaking Task**

In this task participants were presented with a hypothetical situation with which they were confronted (Appendix XX). The chosen scenario describes someone that has to defend themselves to the police after being wrongly accused of shoplifting. Participants were read a description of this situation and then told that they would have 2 minutes to construct a response to the event (Appendix XXI). Participants were then instructed to deliver a 3 minute monologue describing how they might react if they were actually confronted by the situation in real life. Participants were told that their speech would be video recorded and were asked to talk for the entire time, focussing on their feelings and emotions surrounding the situation. Cardiovascular responsivity was measured continuously throughout the 2 minute preparation time and 3 minute speech time.

The speech task is designed to initiate a moderate stress response and has been used with a number of patient and healthy groups to induce significant elevations in blood pressure, heart rate, stroke volume, cardiac output, total peripheral resistance,

heart rate variability, IL-6 and increases in subjective ratings of stress, anger and irritability (Sheffield et al, 1998; Ketterer et al, 2000; Costello et al, 2002).

### **4.2.3 Measures**

#### ***4.2.3.1 Background Measures***

During a structured interview the number of cigarettes smoked per day, hours of exercise undertaken in a four week period and units of alcohol consumed in a typical week were recorded.

#### ***4.2.3.2 Measurements of Student's Adiposity***

Body weight was measured to the nearest 0.1 kg with participants in underwear, and height was measured to the nearest 0.1 cm. Waist circumference was measured horizontally midway between the lowest rib and iliac crest. Hip circumference was measured as the widest part in the gluteal region. Body mass index (BMI) was also calculated as weight in kilograms divided by height in metres squared. Waist-hip ratio (WHR) was calculated by dividing waist by hip circumference.

#### ***4.2.3.3 Task and Mood Ratings***

Each stress task was rated for level of difficulty, anxiety, stress, control, involvement and instruction clarity using a seven point Likert scale (Task Appraisal Questionnaires 1 & 2; Appendix X). A similar seven-point scale measuring subjective stress, relaxation and anxiety was also administered during the rest and post-task periods (Rest Questionnaires 1-4; Appendix XI). Scores on both the Rest and Task Appraisal Questionnaires ranged from 1 = *no stress* to 7 = *very high stress*.



#### **4.2.4 Cardiovascular Measures**

Heart rate, heart rate variability and pre-ejection period were assessed by impedance cardiography using the VU-AMS, as described in section 2.2.4.3. Measurements were taken continually and then averaged during the 5 minute rest, task and recovery periods.

Diastolic and systolic blood pressure was measured using the Portapres Model-2 device (TNO-TPD Biomedical Instrumentation, Amsterdam, Holland). The Portapres system comprises a control unit, front-end unit, two finger cuffs, height correction unit, AC adapter and interface cable. The participant is fitted with two small cuffs attached to the control unit on the middle and ring finger of their non-dominant hand. Each finger cuff is then pressurised and inflated for alternate 30 minute periods to continuously read blood pressure on a beat-to-beat basis. The 5 minute rest, task and recovery periods are then marked using an event button on the control unit. This is later downloaded using personal computer compatible Beatscope software. The Portapres device also allows for adjustment of participant weight, age, height and gender. Data for this study were collected continuously and then averaged over 5 minute intervals during time of interest.

#### **4.2.5 Cortisol Assays**

In the laboratory, salivary cortisol samples were collected using Salivette devices (Sarstedt, Inc. Leicester, UK) at regular 15 minute intervals in order to assess the neuroendocrine response to acute mental stress. Participants were instructed to gently chew on the cotton swab for 120 seconds on eight occasions throughout the experimental procedure. Samples were stored at -30 °C and after defrosting were centrifuged at 3000 rpm for five minutes. 100 µl of supernatant was then used for

duplicate analysis involving a time-resolved immunoassay with fluorescence detection (Dressendörfer, 1992). All assays were carried out by Clemens Kirschbaum and colleagues at Technical University of Dresden.

#### **4.2.6 Cytokine Assays**

Blood was drawn using a 21-gauge butterfly needle into vacutainer tubes containing EDTA as anticoagulant. Whole blood samples (10 ml) were centrifuged immediately at 1250 xg for 10 min at room temperature. Plasma was removed and frozen at -80 °C until analysis. IL-6 was measured using high sensitivity two-site ELISAs from R and D Systems (Oxford, UK). The limit of detection of the IL-6 assay was 0.09 pg/ml, with intra- and inter-assay coefficients of variation of 5.3 % and 9.2 %. All assays were carried out by Dr. Brydon at University College London.

#### **4.2.7 Procedure**

An outline of the procedure is given in Figure 4.1. Participants were individually tested in a purpose-built temperature controlled laboratory. Sessions began at 12:30 pm with participants being given a high carbohydrate, low protein meal (to control for the effects of protein and carbohydrate-increased cortisol release which may mask the task response). A brief structured interview examining participant's current state of health and previous day's events was then administered. A series of standardised physical measurements to assess adiposity were also completed. These included measurement waist to hip ratio and body mass index. The VU-AMS was then fitted, as described in Figure 2.1. A 21 gauge butterfly cannula was also inserted into the participant's non-dominant lower arm, before the Portapres-2 device was attached and the first saliva sample taken. A manual blood pressure reading was taken to ensure the Portapres-2 device is working effectively.

Participants were then left to relax and familiarise themselves with the equipment for 30 minutes. Within the last 5 minutes of this rest period continuous VU-AMS and Portapres readings were recorded before participants complete the first rest questionnaire and second saliva sample. Following this the baseline blood sample was drawn. Participants then performed the two mental stress tasks, completing a task impact questionnaire and saliva sample after each. VU-AMS and Portapres recordings were taken throughout the task period, and a first post-task blood sample was drawn.

Participants were then instructed to relax for the next 90 minutes. During this recovery period, salivary cortisol samples and rest questionnaires were completed at 15, 30, 45 and 90 minutes post-task. Five minutes of continuous VU-AMS / Portapres recordings were also taken during these times and two post-task blood samples were drawn at 45 and 90 minutes post task.

#### **4.2.8 Family History Measures**

Family history health status was provided by participants' parents. Contact details were obtained, and a cover letter and brief questionnaire was posted to at least one blood-related parent for each participant (Appendix XXII). Factors included in the questionnaire were based on recommendations by Higgins et al (1996). Parents were asked to provide details of their own age, weight, height, waist circumference and smoking history. They were also asked whether they had ever been told by a doctor that they had high blood pressure, heart disease, diabetes or high cholesterol; age of diagnosis was subsequently noted. Parents provided details of the health status of participants' grandparents, including history of diabetes, heart disease, high cholesterol or high blood pressure. Questionnaire responses were rated so that an overall risk of future cardiovascular disease, hypertension and raised adiposity was determined for

each individual taking into account, grandparent's and parent's current and previous health status.

In total, 88 (85.4 %) participants provided contact details for both parents with 15 (14.6 %) participants providing contact details for just one parent. A total of 191 questionnaires were posted and 156 were returned, yielding a response rate of 81.7 %. Of these returned questionnaires, family history data from at least one parent was available for 88.3 % of the sample, while 63.1 % of participants had family history data available from both blood-related parents. The offspring of parents who did not respond to the questionnaire had similar waist to hip ratio, body mass index, heart rate, heart rate variability, systolic and diastolic blood pressure baseline, reactivity and recovery levels, in comparison with those who did return the parent questionnaire.  $\chi^2$  tests also revealed no gender difference between students whose parents responded and those who did not; however, the offspring of parents who did not return the questionnaire had significantly shorter pre-ejection periods at baseline and during the first stress task ( $t = 2.34$ ,  $p = .021$ ;  $t = 2.06$ ,  $p = .042$ ).

All parents who returned their questionnaire provided information about one set of participant's grandparents. From a potential of 412, details were gained for 316 (76.7 %) grandparents, and 88.3% of participants had information from at least two grandparents.

#### ***4.2.8.1 Family History of Cardiovascular Disease Score***

When devising the cardiovascular disease risk score, study sample characteristics, and the recommendations by Silberberg et al (1999) for family history score of common disease were considered. First, it is recommended that the risk profile of the entire family should be considered. Based on the design of Treiber et al (1993), it was decided, that evidence of heart disease, high blood pressure, high cholesterol or

diabetes in parents and grandparents would be used to determine overall cardiovascular disease risk. Second, it is recommended that scores should be robust to family size. The majority of previous studies have favoured the use of a count of the number of parents with a disease, versus the number of parents without the disease. However, due to the fact that a number of questionnaires were only returned by one parent, and this particular study is interested in numerous cardiovascular disease risk factors it was decided that a count of the number of risk factors, instead of a count of number of relatives would be used. Third, Silberberg et al (1999) recommends that a family history score should consider the relationship of the relatives; therefore the number of cardiovascular-related disorders of parents was given double the weight of the cardiovascular score in grandparents.

In summary, family history of cardiovascular disease was calculated by summing the total number of cardiovascular-related risk factors for parents (multiplied by 2), with the total number of cardiovascular-related risk factors for grandparents. For example, if a student's mother had heart disease and diabetes, and father had no history of any of the four disorders the student would receive a parental score of 4 (2 disorders multiplied by 2). If the same student had two grandparents with high cholesterol, the student would be given a grandparent score of 2 making their total family history score 6 (4 from parents scores, 2 from grandparents scores). Good individual variability in cardiovascular disease score totals was obtained. The number of total parental and grandparent cardiovascular-related risk factors ranged from 0 (no family history of cardiovascular disorders) to 15 (high family history of cardiovascular disorders), of a possible score of 32. The total parent and grandparent scores were then dichotomised using a conservative classification criterion so that two groups with a divergent family history were created. Only two groups were used as the young sample meant that very

few participants had two parents who suffered from more than one disorder. A subject was defined, as having a negative family history of cardiovascular disease if neither parent nor any of the four grandparents had evidence of cardiovascular disease risk factors; or if the four grandparents collectively reported just one cardiovascular disease risk factor (i.e. the student's original score was 0 or 1). Conversely, a subject was defined as having a positive family history of cardiovascular disease if the total number of cardiovascular disease-related risk factors presented by both parents and grandparents exceeded two (i.e. the student's original score was 2 or more). In total, 75 participants had a positive family history of cardiovascular disease compared to 16 participants with a negative family history of cardiovascular disease. Participants who had only one out of their two parents respond had the non-responding parent classified as negative; while participants who had neither parent respond were eliminated from the analyses ( $n = 12$ ).

#### ***4.2.8.2 Family History of Hypertension Score***

Family history of hypertension was created in a similar way. A participant was defined as having a positive history of hypertension if at least one parent or at least two grandparents reported a history of raised blood pressure. A participant was defined as having a negative history of hypertension if neither of their parents, or one (or none) of their four grandparents reported high blood pressure. In total, 64 participants had a negative history of hypertension, while 27 participants had a positive family history of hypertension.

#### ***4.2.8.3 Parental Adiposity Score***

Parental adiposity was classified separately for mother and father's BMI and separately for mother's and father's waist measurement also. Scores for both BMI and waist were divided into tertiles so that group comparisons with student reactivity could

be made. Mothers classified in the low tertile had a BMI below 23.33 ( $n = 20$ ) and a waist measurement less than 73.6 cm ( $n = 25$ ); while mothers in the high tertile had a BMI that exceeded 25.83 ( $n = 23$ ) and waist measurements greater than 81.2 cm ( $n = 21$ ). Similarly, fathers classified in the low tertile had a BMI below 24.18 ( $n = 23$ ) and a waist measurement less than 86.3 cm ( $n = 22$ ); while fathers in the high tertile had a BMI that exceeded 27.87 ( $n = 23$ ) and waist measurements greater than 96.5 cm ( $n = 16$ ).

#### 4.2.9 Data Reduction

Cardiovascular data (systolic blood pressure, diastolic blood pressure, heart rate, heart rate variability and pre-ejection period) were averaged into seven 5 minute trial periods (baseline, task 1, task 2, 10-15 min post-task, 25-30 min post-task, 40 45 min post-task and 85-90 min post-task). Data were screened for technical errors, equipment failure and for cardiovascular responses which fell outside possible parameters.

Participants with erroneous data were subsequently removed from the analyses. Results were based, on the full sample ( $n = 103$ ) for pre-ejection period and heart rate analyses, 85 participants for diastolic blood pressure, 87 for systolic blood pressure and 98 participants for heart rate variability analyses; the missing blood pressure data were due largely to technical problems with the Portapres device, which was functioning incorrectly for approximately 3 weeks. Cardiovascular reactivity (including heart rate, systolic and diastolic BP and PEP) was defined as task value minus baseline level for each of the cardiovascular parameters during tasks 1 and 2; higher scores indicate greater task-reactivity. Heart rate variability reactivity was defined as task value minus baseline level; with larger decreases in HRV indicating greater stress response. Cardiovascular recovery was defined as values 45 minutes post-task minus baseline level for each cardiovascular parameter; greater scores indicate poorer task-recovery.

Since this method does not take account of task responses, task response was added as a covariate. I recognise that that this investigation operationalised task recovery differently from Study 1, however, both methods are accepted in the literature (Linden et al, 1997). Study 1 calculated recovery minus task maximum (controlling for baseline) due to the small magnitude of task response which would not have differentiate individuals very effectively using the alternative method.

Cortisol samples with concentrations less than 1 nmol/l or more than 65 nmol/l were not analysed because this indicates insufficient saliva volume or sample contamination (n = 5). Samples from participants who fainted during the procedure were also excluded from the analyses (n = 6). Analyses of area under the curve revealed that these participants had significantly higher cortisol concentrations as a result of syncope ( $t = 7.39$ ,  $p = .001$ ). Cortisol results were based, on 92 participants. Similarly, it was not possible to gain plasma samples from 11 participants. Analyses of IL-6 concentrations were based on data from 92 participants.

There were eight cortisol samples in total. Sample 1 was discarded as this was used for practice purposes only. Sample 2 was, therefore, used as the baseline level. Komogorov-Smirnov test revealed that cortisol samples were heavily skewed ( $p = .001$ ) so transformations using square root were used for all samples. Inspection of the data indicated that participants varied greatly in the time course of cortisol response to tasks. Twenty-eight participants (30.4 %) showed a peak in cortisol with sample 3, 5 (5.4 %) with sample 4, 26 (28.3 %) with sample 5, 6 (6.5 %) with sample 6 and 7, and 21 (22.8 %) with sample 8. A peak in cortisol concentration at the end of the study procedure was found. This may not have been due to the effects of the stressor; so this sample was omitted from repeated measures analysis. Cortisol responses were analysed, by



selecting the highest post-task value from samples 3 to 6. Reactivity was defined as the change between this value and the baseline level (Sample 2).

Komogorov-Smirnov test revealed that none of the four IL-6 samples were normally distributed ( $p = .001$ ). Square root transformations were used for all four samples. There was less variation in IL-6 response to tasks, with 65 % of participant's IL-6 concentrations peaking 90 minutes post-task. IL-6 reactivity was therefore defined as the difference between this sample (Sample 4) and baseline (Sample 1).

#### 4.2.10 Statistical Analyses

Comparison of men and women's background characteristics were made using univariate analysis of variance.  $\chi^2$  calculations were used for comparison of smoking status by gender. Comparisons of mother and father's background characteristics were made using univariate analysis of variance (ANOVA).  $\chi^2$  test was used for comparison of mothers' and fathers' smoking status and incidence of parental and grandparent cardiovascular risk factors. The overlap between classification of hypertension, cardiovascular disease and adiposity risk was presented in terms of group percentages. Task appraisals were compared for both tasks using independent t-tests, with gender as the between-subject factor.

Analysis of covariance (ANCOVA) was used to assess baseline physiological differences between men and women (covariates included student's smoking status, waist to hip ratio and body mass index). Paired samples t-tests were used to compare task impact ratings between the two tasks; while independent samples t-tests were used to assess gender differences in task ratings. Task ratings were also compared by family history risk using independent samples t-tests.

Physiological profiles over the session were assessed using repeated measures ANOVA, with gender as the between-subject factor and trial as the within-subject

factor. Two trial periods were analysed for cortisol, four for IL-6 concentrations, seven for subjective stress ratings and six for cardiovascular measures. It was decided to exclude the 90 minute post-task reading for cardiovascular measures as there was a significant elevation in cardiovascular response at this time, possibly due to restlessness of the participants at the end of the study. *Post hoc* comparisons between individual values were made using Tukey's LSD test.

Multiple linear regressions, controlling for gender, were used to analyse student adiposity and baseline physiological measures. Parental adiposity and baseline physiological measures were also assessed using linear regression adjusted for gender and student's own adiposity. Univariate analysis of variance was used to compare baseline physiological measures by family history of cardiovascular disease, controlling for gender and student's body mass index and waist to hip ratio. Univariate ANOVA was also used to compare family history of cardiovascular disease and student's weight, controlling for gender.

The relationship between physiological responsivity and student's adiposity (waist to hip ratio and body mass index) was assessed using repeated measures ANOVA with gender and adiposity (split into tertiles) as the between-subject factor and trial as the within-subject factor. The Greenhouse-Geisser correction for degrees of freedom was applied when the assumption of sphericity was violated, and *p* values were adjusted accordingly. Follow-up analyses assessing student adiposity and physiological reactivity to and recovery from the task were made using univariate analysis of covariance, controlling for gender, smoking status and baseline physiological levels.

The relationship between physiological responsivity and parental adiposity (mother and father's body mass index and waist measurement) was also assessed using repeated measures ANOVA with gender and parental adiposity (split into tertiles) as the

between-subject factor and trial as the within-subject factor. Follow-up analyses assessing parental adiposity and physiological reactivity to and recovery from the task were made using multiple linear regressions controlling for student's own gender, smoking status, adiposity (waist to hip ratio or body mass index) and baseline levels.

Repeated measure analysis was used to assess the relationship between physiological reactivity, family history of hypertension, and then family history of cardiovascular disease. Gender and family history were used as between-subject factors, with trial as the within-subject factor. Univariate analysis of variance, with family history and gender as the between-subjects factors were used to assess physiological reactivity to and recovery from the two tasks, controlling for student's own smoking status, adiposity (BMI and WHR), baseline levels.

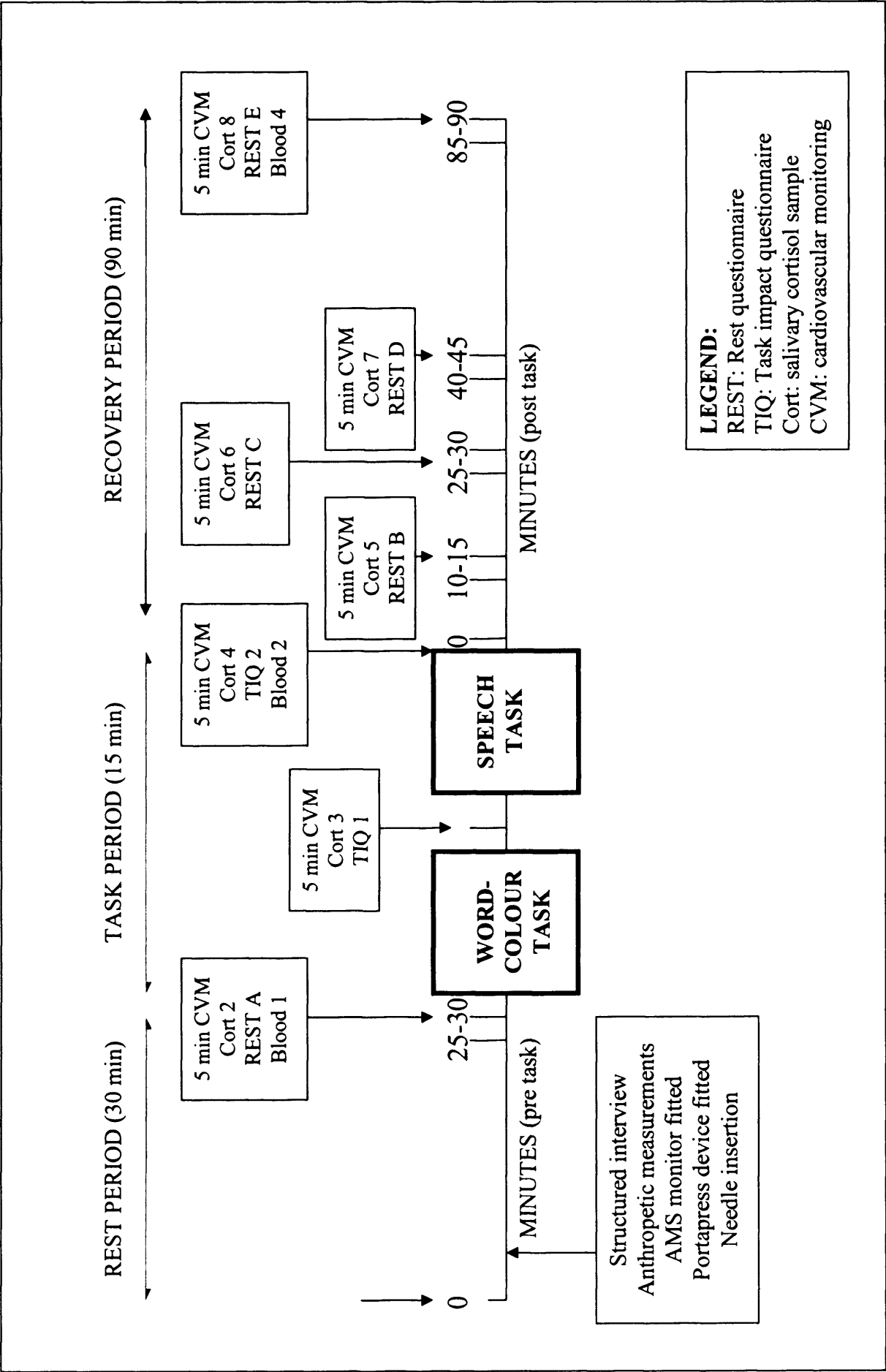


Figure 4.1: Study 3 Procedure Outline

## 4.3 Results

Tables and Figures to accompany this section start on page 239

### 4.3.1 Student Sample Characteristics

The characteristics of the student sample are summarised in Table 4.2. In general, the sample was fit and healthy; only 15.5 % of the total sample smoked, average hours of exercise per week were above the government's recommended level, and alcohol consumption was low. Gender comparisons showed that men and women were of a similar age, had comparable body mass indexes (BMI), drank a similar amount of alcohol per week and partook in similar amounts of exercise. A similar number of men and women were smokers and men had significantly larger waist to hip ratios (WHR) ( $t = 4.38$ ,  $p = .001$ ) than women.

### 4.3.2 Parent Sample Characteristics

Characteristics of the parent sample are summarised in Table 4.3. Student's parents were generally young and healthy; few of them smoked (12.7 %) and half reported at least one cardiovascular-related condition. Gender comparisons show that mothers were significantly younger ( $t = 3.60$ ,  $p = .001$ ) and had smaller waist circumferences ( $t = 5.23$ ,  $p = .001$ ) than fathers; while body mass index was only marginally smaller in mothers compared with fathers ( $t = 1.96$ ,  $p = .052$ ). A similar number of mothers and fathers were smokers, and incidence of high blood pressure, heart disease and diabetes did not differ by gender.  $\chi^2$  analysis revealed that fathers had a greater incidence of high cholesterol than mothers ( $\chi^2 = 4.34$ ,  $p = .037$ ), but comparison of overall incidence of cardiovascular-related conditions did not differ by gender.

### 4.3.3 Grandparent Sample Characteristics

From the 158 parents who returned the questionnaire, details were provided for 309 grandparents (of a possible 412). In general, the student's grandparents were relatively healthy, with just 157 (50.8 %) of them reporting one or more cardiovascular-related disorder. 71 (23.4 %) of the grandparents whose details were recorded, were reported to suffer from heart disease, 38 (12.4 %) suffered from high cholesterol, 92 (30.2 %) had high blood pressure, and 45 (14.6 %) suffered from diabetes. No difference in condition incidence was observed between grandmothers and grandfathers using  $\chi^2$  analysis. Similarly, no difference between condition incidences was observed for maternal and paternal grandparents.

### 4.3.4 Overlap between Family History Categorisation

Family history scores were calculated for risk of cardiovascular disease, risk of hypertension and risk of obesity (according to mother and father's BMI and waist measurement). Of the 75 participants classified with a positive risk of cardiovascular disease, 27 (36 %) had a positive risk of hypertension. This overlap was 25 % for participants in the negative cardiovascular disease group and negative hypertension risk group. Positive and negative family history of cardiovascular disease classification also overlapped with the highest and lowest tertiles of adiposity. That is, 58.5 % of those classified with a positive risk of cardiovascular disease had mothers in the highest tertile of waist measurement; while there was a 32 % overlap for negative cardiovascular disease and low adiposity risk. The group overlap for father's waist tertiles and cardiovascular risk was 90.9 % for participants in the positive cardiovascular disease and large waist tertile group. This was 75 % for participants with a negative or low risk of cardiovascular disease and adiposity. Similarly, 96.4 % of participants with a

positive family history of cardiovascular disease had a high risk of adiposity (defined by mother's BMI). This overlap was 91.7 % for participants with a negative or low risk of cardiovascular disease and adiposity. Lastly, 95.7 % of participants with a positive family history of cardiovascular disease had fathers with a BMI in the highest tertile. 85.7 % of participants with a negative family history of cardiovascular disease had fathers with a low BMI.

### **4.3.5 Physiological Baseline Measures**

#### **4.3.5.1 Gender**

Baseline cortisol level averaged 2.33 nmol/l ( $\pm .60$ ) and did not differ by gender. Similarly baseline heart rate, heart rate variability, pre-ejection period and IL-6 concentrations did not differ by gender. Mean diastolic BP averaged 65.27 mmHg at baseline and this did not differ by gender, however men's systolic BP was on average 11.4 mmHg higher than women's systolic BP at baseline ( $F_{1,82} = 16.16$ ,  $p = .001$ ), when adjusting for student's smoking status, WHR and BMI (Table 4.2).

#### **4.3.5.2 Student Adiposity**

Linear regression showed that participants with larger body mass indices had higher resting diastolic blood pressure ( $B = .74$ , C.I. .28 to 1.21,  $p = .002$ ) and pre-ejection period ( $B = .68$ , C.I. .19 to 1.17,  $p = .007$ ) than those with a lower BMI. Waist to hip ratio was not related to any baseline physiological measures, when controlling for gender. There was no association of baseline IL-6 with student's own adiposity.

#### **4.3.5.3 Parental Adiposity and Family History of Cardiovascular Disease**

Linear regression analyses revealed that baseline IL-6 was strongly associated with father's body mass index ( $B = .002$ , C.I. .005 to .027,  $p = .006$ ), but not mother's

positive family history of cardiovascular disease had a high risk of adiposity (defined by mother's BMI). This overlap was 91.7 % for participants with a negative or low risk of cardiovascular disease and adiposity. Lastly, 95.7 % of participants with a positive family history of cardiovascular disease had fathers with a BMI in the highest tertile. 85.7 % of participants with a negative family history of cardiovascular disease had fathers with a low BMI.

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BMI, when controlling for own BMI. This factor accounted for 11.6 % of the variance in baseline IL-6 concentration. Similarly, baseline IL-6 was marginally associated with fathers but not mother's waist measurement ( $B = .24, p = .069$ ) when controlling for student's own adiposity. None of the cardiovascular (diastolic BP, systolic BP, HR, HRV, PEP) or neuroendocrine (cortisol) baseline values were associated with mother's or father's BMI or waist measurement, when adjusting for own adiposity. Baseline physiology of subjects with a positive family history (FH) of cardiovascular disease (CVD) did not differ from those who had a negative risk of cardiovascular disease.

#### 4.3.6 Response to Study Protocol

Physiological and subjective responses to the trial are summarised in Table 4.4. Of particular interest was participant's response to and recovery from the two mental stress tasks. Reactivity scores were calculated by subtracting baseline physiological levels from those experienced during the tasks. Similarly, recovery scores were calculated by subtracting the third recovery period value (45 min post-task) from the baseline level (as outline in Section 4.2.9). Responsivity levels for male and female participants are outlined in Table 4.6. It should be emphasised that individual differences in cardiac responses were substantial for both tasks. For example, blood pressure responses for task 1 ranged from -17.78 to + 65.02 mmHg for systolic BP and -12.07 to + 32.95 for diastolic BP; while ranges of recovery response were equally as large. There were also large between-task differences in reactivity. Paired-samples *t*-tests comparing cardiovascular reactivity to task 1 and 2 revealed that diastolic BP ( $t = -10.66, p = .001$ ), systolic BP ( $t = -10.30, p = .001$ ), heart rate ( $t = -9.05, p = .001$ ) and PEP reactivity ( $t = 6.35, p = .001$ ) were all significantly higher for the second task; however, there was no difference in HRV reactivity between the two tasks. It is usually recommended that an average task score is analysed, however, because of the larger

reactions to the second task, responsivity to the two tasks was analysed separately. A more restricted range was found for cortisol and IL-6 reactivity with only a 10 % increase in task-related cortisol levels, and an 8 % rise in stress-induced IL-6 concentrations present; the possible consequence of this is discussed later.

#### **4.3.6.1 Subjective Task Ratings**

The 7-point Task Impact Questionnaires revealed that both tasks led to feelings of stress, poor performance, a lack of control and high involvement (Table 4.5). Participants reported that the Stoop task was significantly more difficult ( $t = 7.10$ ,  $p = .001$ ) than the speech task. Participants also felt less involved ( $t = -11.37$ ,  $p = .001$ ), less in control ( $t = -10.09$ ,  $p = .001$ ) and less relaxed ( $t = -3.0$ ,  $p = .003$ ) during the Stroop task. Similarly, participants thought they had performed more poorly on this task ( $t = -11.37$ ,  $p = .001$ ) compared with the Speech test. Female participants reported that they found the speech task more difficult ( $t = -2.08$ ,  $p = .040$ ) and felt less relaxed ( $t = -2.13$ ,  $p = .036$ ) than male subjects.

#### **4.3.6.2 Subjective Stress Ratings**

Repeated measures analyses of variance showed that participant's subjective stress ratings changed significantly over the 7 trials ( $F_{6,606} = 162.43$ ,  $p = .001$ ). On administration of the two tasks, stress ratings rose sharply ( $p = .001$ ) before returning quickly to levels similar to baseline during the recovery session ( $p = .001$ ). Responses for men and women were similar throughout the procedure.

#### **4.3.6.3 Blood Pressure**

A significant main effect of trial was discovered for mean diastolic blood pressure ( $F_{5,410} = 77.39$ ,  $p = .001$ ). *Post hoc* tests indicate a significant increase in blood pressure during the first task ( $p = .001$ ), followed by a further increase in blood pressure

from task 1 to task 2 ( $p = .001$ ). A sharp decline is then observed with the onset of the recovery phase ( $p = .001$ ), and diastolic blood pressure was significantly greater than baseline during the final recovery point ( $p = .001$ ). A time-gender interaction ( $F_{5,410} = 2.99$ ,  $p = .024$ ) but no main effect for gender was found (see Table 4.4.). Univariate analysis of covariance revealed a significant main effect of gender for both stress tasks (task 1,  $F_{1,79} = 6.64$ ,  $p = .012$ ; task 2,  $F_{1,79} = 5.61$ ,  $p = .020$ ), when controlling for BMI, WHR and smoking status; indicating that men were more reactive to both tasks. No gender difference was found for diastolic BP recovery.

A similar pattern was observed for systolic blood pressure. Mean systolic BP differed over the 6 trials ( $F_{5,420} = 75.45$ ,  $p = .001$ ). *Post hoc* analysis revealed that there was a significant increase in blood pressure in response to task 1 ( $p = .001$ ). A significant increase was then seen from task 1 to task 2 ( $p = .001$ ), followed by a significant decline during the recovery period ( $p = .001$ ). Again systolic blood pressure did not return to baseline within the recovery period and was significantly higher than baseline at the last recovery test point ( $p = .013$ ). A time-gender interaction ( $F_{5,410} = 8.62$ ,  $p = .001$ ) and main effect of gender were also present ( $F_{1,84} = 28.97$ ,  $p = .001$ ), indicating that men had higher systolic BP during the procedure. Univariate ANCOVA revealed that these gender effects were present during task 1 ( $F_{1,81} = 3.99$ ,  $p = .049$ ) and task 2 ( $F_{1,81} = 5.40$ ,  $p = .023$ ) but not task recovery ( $F_{1,81} = 3.23$ ,  $p = .076$ ), when controlling for WHR, BMI and smoking status.

#### **4.3.6.4 Heart Rate**

A significant main effect of trial was discovered for heart rate ( $F_{5,505} = 163.78$ ,  $p = .001$ ). *Post hoc* tests suggest a sharp rise in heart rate during task 1 ( $p = .001$ ), followed by an increase in heart rate from task 1 to task 2 ( $p = .001$ ). A quick return to baseline during the first recovery period was also observed ( $p = .001$ ). Heart rate

remained at a level similar to baseline for the remaining three recovery periods. No trial-gender interaction or main effect of gender was present (Table 4.4).

#### **4.3.6.5 Heart Rate Variability**

Mean HRV differed over the 6 trials ( $F_{5,475} = 46.25$ ,  $p = .001$ ). *Post hoc* analyses suggested a sharp fall in HRV during task 1 ( $p = .001$ ), followed by a decrease in HRV from task 1 to task 2 ( $p = .001$ ). A return to baseline levels was then seen during the first and subsequent recovery periods ( $p = .001$ ). No gender-trial interaction or main effect of gender was observed (Table 4.4).

#### **4.3.6.6 Pre-Ejection Period**

A main effect of trial was found for PEP ( $F_{5,475} = 79.78$ ,  $p = .001$ ). *Post hoc* tests indicate that pre-ejection time was significantly shorter during the two tasks than at baseline ( $p = .001$ ) and that PEP remained below baseline levels at all three recovery periods ( $p = .001$ ). No gender by trial or main effect of gender was observed (Table 4.4).

#### **4.3.6.7 Cortisol**

Paired samples t-test revealed a significant increase in cortisol from baseline to maximum post-task cortisol concentration ( $t = 4.0$ ,  $p = .001$ ). Repeated measures analysis of variance indicated that no time-gender interaction or gender main effect was present (Table 4.4).

#### **4.3.6.8 Interleukin-6**

Plasma IL-6 concentrations changed significantly during the experimental procedure ( $F_{3,270} = 19.99$ ,  $p = .001$ ). *Post hoc* tests reveal that IL-6 increased significantly from baseline in a linear fashion at all three time points (immediately post

task,  $p = .017$ ; 45 min post-task,  $p = .001$ ; 90 min post-task,  $p = .001$ ). A time-gender interaction was found ( $F_{3,270} = 4.66$ ,  $p = .021$ ) but no gender main effect was observed (Table 4.4; Figure 4.2). Estimated means suggest that females had greater increases in IL-6 production between baseline and 90 min post-task than males (.95 and .87, respectively). Univariate analyses of covariance also indicate that females had significantly greater IL-6 change scores than male participants when controlling for baseline IL-6 concentrations, smoking status, BMI and WHR ( $F_{1,86} = 10.01$ ,  $p = .002$ ).

### 4.3.7 Student Adiposity and Physiological Responsivity

#### 4.3.7.1 Blood Pressure Responsivity and Student Adiposity

Analyses of student's systolic BP and waist to hip ratio (divided into tertiles) revealed a three-way interaction between trial, gender and WHR ( $F_{10,400} = 3.32$ ,  $p = .007$ ). When the sample was split by gender, analyses indicated that this three-way interaction between WHR and trial was accounted for by male participants ( $F_{2,21} = 3.68$ ,  $p = .043$ ) (Figure 4.3). However, univariate analyses of covariance (ANCOVA) revealed that there was no difference between male or female WHR and diastolic BP reactivity to either task 1 or task 2. No interactions involving BMI were discovered.

Analyses of diastolic blood pressure and student's WHR revealed a significant three-way gender, trial and WHR interaction ( $F_{10,390} = 2.56$ ,  $p = .014$ ). When the sample was split by gender, analysis indicated that this three-way interaction between WHR and trial was accounted for by male participants ( $F_{10,105} = 2.89$ ,  $p = .022$ ) (Figure 4.4). Furthermore, repeated measures analyses revealed a significant linear relationship between diastolic BP reactivity during tasks 1 and 2 and WHR ( $F_{2,21} = 4.70$ ,  $p = .021$ ). This indicates that male students with a higher WHR had a greater reaction to the task than those with a medium and low WHR (high WHR = 87.27 mmHg; medium WHR =

81.86 mmHg; low WHR = 77.26 mmHg). No BMI by trial or three-way interaction was present.

#### **4.3.7.2 Heart Rate Responsivity and Student Adiposity**

A WHR by gender interaction was present for student's heart rate over the study session ( $F_{2,97} = 5.37$ ,  $p = .006$ ). A three-way interaction was also found for WHR, gender and trial ( $F_{10,485} = 2.32$ ,  $p = .050$ ). When the sample was split by gender, *post hoc* analysis revealed that the main effect of WHR was only present for male participants ( $F_{2,31} = 5.08$ ,  $p = .012$ ). Means indicate that this difference was due to low heart rate levels of the low WHR group throughout the session (low WHR = 65.4 bpm; medium WHR = 75.0 bpm; high WHR = 77.0 bpm). A marginal trial by WHR interaction was present for male subjects ( $F_{10,155} = 1.83$ ,  $p = .061$ ), with sphericity assumed. Univariate analyses indicated that male and female heart rate reactivity to, and recovery from the tasks did not differ by WHR or BMI. No interactions or main effect of BMI was found.

#### **4.3.7.3 Heart Rate Variability Responsivity and Student Adiposity**

Analyses of HRV by WHR tertiles during the study session revealed no significant trial or three-way interaction. No main effect of WHR was found. Univariate ANCOVA revealed no difference between HRV task reactivity or recovery and WHR. In contrast, analysis of HRV and BMI tertiles revealed a significant BMI by trial interaction ( $F_{10,455} = 2.25$ ,  $p = .045$ ). This appears to be due to a low response in the small BMI group. HRV baseline level for this group averaged 48.5, this then decreased to a minimum of 36.1 during task 2, before returning to 51.9 at the end of the session. In contrast, HRV levels in the large and medium BMI group (respectively) began at 60.6 and 63.3 before dropping to 39.3 and 36.5 during task two, these levels

then return to 61.8 and 60.7 on completion of the session. Univariate analyses of covariance revealed that HRV to or recovery from the stress tasks did not differ by WHR or BMI.

#### ***4.3.7.4 Pre-Ejection Period Responsivity and Student Adiposity***

No significant main effects or interactions of WHR or BMI were discovered.

#### ***4.3.7.5 Cortisol Reactivity and Student Adiposity***

Analyses revealed that cortisol did not differ by WHR or BMI over trial. In addition, no three-way interaction or main effects of WHR or BMI were present. Univariate ANCOVA also suggests that cortisol reactivity to the tasks did not differ by student adiposity.

#### ***4.3.7.6 Interleukin-6 Reactivity and Student Adiposity***

No significant main effects or interactions of WHR or BMI were found.

#### ***4.3.7.7 Summary of Student Adiposity and Physiological Responsivity***

In summary, heart rate, diastolic and systolic blood pressure differed by waist to hip ratio over the session, but the effects on blood pressure changes were only found for male participants. Follow-up analysis examining specific sections of the study session revealed that diastolic blood pressure task reactions differed according to WHR. Heart rate variability also differed according to BMI, and this was due to the low responsivity of the low BMI group; the effect did not reach significance during task reactivity or recovery. In addition, student adiposity, as defined by WHR and BMI, was not related to neuroendocrine or cytokine reactivity to the tasks.

### 4.3.8 Parental Adiposity and Physiological Responsivity

Data were collected on students' mother's and father's body mass indices and waist circumferences. For repeated measures analysis these measures were divided into tertiles. Correlational analyses revealed that student's waist circumference and BMI were not associated with either mother's or father's waist or BMI measurement (all correlations  $< .20$ ). In addition, analyses indicated that student's subjective task and stress ratings did not differ according to parental adiposity.

#### 4.3.8.1 Blood Pressure Responsivity and Parental Adiposity

Analyses of student's systolic BP by mother's body mass index revealed no main effect of BMI, time by BMI interaction, or three-way interaction. Similarly, analyses of systolic BP and mother's waist measurements revealed no main effect of waist, time by waist or three-way interaction. Univariate ANCOVA showed that systolic BP reactivity to, and recovery from, the two stress tasks did not differ according to mother's BMI or WHR when controlling for student's own baseline blood pressure, smoking status and BMI or waist measurement.

Analyses of student's systolic BP divided by tertiles of father's body mass index revealed a time by BMI interaction with sphericity assumed ( $F_{10,255} = 2.05$ ,  $p = .029$ ). This interaction was marginally significant with the Greenhouse-Geisser correction applied ( $F_{10,255} = 2.05$ ,  $p = .077$ ) (Figure 4.5). Univariate ANCOVA showed a significant quadratic contrast effect for reaction to the second task ( $F_{2,51} = 5.75$ ,  $p = .026$ ). Mean reactivity change scores for the second task, adjusted for student's own baseline blood pressure, smoking status, gender and BMI revealed that the effect was due to the low response in the 'medium BMI group' (large BMI = 27.5 mmHg; medium



BMI = 14.8 mmHg; small BMI = 27.7 mmHg). No differences between fathers' BMI were discovered for task 1 reactivity or task recovery.

Similarly, analyses of systolic BP by father's waist measurement revealed a trial by waist interaction with sphericity assumed ( $F_{10,235} = 2.07$ ,  $p = .028$ ). This was marginally significant with the Greenhouse-Geisser correction applied ( $F_{10,235} = 2.07$ ,  $p = .072$ ). A significant three-way interaction was also found ( $F_{10,235} = 3.41$ ,  $p = .006$ ). When the sample was split by gender, univariate ANCOVA revealed a quadratic contrast effect for fathers' waist circumference and male systolic blood pressure to the first task ( $F = 17.90$ ,  $p = .010$ ). Mean change scores, adjusted for student's own baseline blood pressure, smoking status and waist measurement, indicate that this effect was not linear, due to the low response level in the 'medium waist group' (large waist = 18.7 mmHg; medium waist = -1.9 mmHg; small waist = 21.2 mmHg). In addition, a linear contrast effect was found for female participants ( $F = 7.02$ ,  $p = .036$ ). This indicated that female participants with the largest systolic response to the first task had fathers with larger waist circumferences, and that females with the smallest systolic reaction to the first task had fathers with the smallest waist circumference (large waist = 10.7 mmHg; medium waist = 8.2 mmHg; small waist = .82 mmHg).

Analyses of diastolic blood pressure by mother's body mass index revealed no trial by BMI, or three-way interaction. Analysis by mother's waist measurement of diastolic BP revealed a marginal gender by waist interaction ( $F_{2,49} = 3.03$ ,  $p = .058$ ). No interactions or main effects of mother's waist were present. Analysis of diastolic BP by father's body mass index revealed no interactions or main effects. However, a three-way interaction between gender, trial and waist measurement was present ( $F_{10,225} = 2.18$ ,  $p = .037$ ). When the sample was split by gender, analyses showed that a significant trial by waist interaction was present only in male participants ( $F_{10,70} = 2.48$ ,  $p = .040$ ).

Univariate ANCOVA revealed a quadratic contrast effect for fathers' waist measurement and male diastolic blood pressure to the second task ( $F = 12.25$ ,  $p = .001$ ). Mean change scores, adjusted for student's own baseline blood pressure, smoking status and waist measurement, indicate that this effect was not linear, again due to the low response level in the 'medium waist group' (large waist = 18.8 mmHg; medium waist = 7.8 mmHg; small waist = 26.8 mmHg). Univariate ANCOVA also revealed no difference between father's waist circumference and diastolic blood pressure in reaction to task 1 or recovery from the tasks.

#### ***4.3.8.2 Heart Rate Responsivity and Parental Adiposity***

Heart rate did not differ by mother's body mass index over the trial. In addition, no three-way interaction or main effect of BMI was discovered. No interactions or main effects of heart rate by mother's waist measurement or father's body mass index were discovered. A waist by trial interaction was present for fathers' waist circumference ( $F_{10,290} = 3.32$ ,  $p = .007$ ); although univariate ANCOVA revealed a quadratic contrast effect for fathers' waist measurement and heart rate reactivity to the second task in men ( $F = 8.51$ ,  $p = .001$ ). Mean change scores, adjusted for student's own baseline heart rate, smoking status and waist measurement, indicate that this effect was due to the low response level in the 'medium waist group' (large waist = 18.4 bpm; medium waist = 7.9 bpm; small waist = 18.4 bpm). Univariate ANCOVA revealed no difference between father's waist circumference and heart rate in reaction to task 1 or recovery from the tasks.

#### ***4.3.8.3 Heart Rate Variability Responsivity and Parental Adiposity***

No main effect or interaction of mother's BMI or waist circumference was present. In addition, no main effect or interactions were found for trial, gender and

father's BMI; although, a BMI by gender interaction was discovered ( $F_{2,59} = 4.15$ ,  $p = .021$ ). *Post hoc* analyses investigating gender separately revealed that this gender by BMI interaction was probably accounted for by a marginal main effect of BMI for male participants only ( $F_{2,18} = 3.47$ ,  $p = .053$ ); however estimated means for the whole sample reveal that during the trial, participants whose fathers had the largest BMI also had higher HRV than the other two groups (large BMI = 72.96; medium BMI = 49.68; small BMI = 37.46). Univariate ANCOVA revealed no difference in relation to fathers' BMI and HRV in reaction to or recovery from the tasks. No main effect or interaction of father's waist circumference was present.

#### ***4.3.8.4 Pre-Ejection Period Responsivity and Parental Adiposity***

No main effects or interactions involving mother's BMI or waist circumference were present in analysis of pre-ejection period. Similarly, analysis revealed no main effect or interactions involving father's BMI. For father's waist measurement no interactions were discovered. Only a main effect of waist was found ( $F_{2,55} = 6.20$ ,  $p = .004$ ); this effect was not linear (large BMI = 123.98; medium BMI = 127.35; small BMI = 118.30). No other effects were discovered for parental adiposity and pre-ejection period.

#### ***4.3.8.5 Cortisol Reactivity and Parental Adiposity***

No significant main effect or interactions were present for mother's body mass index or waist measurement. In contrast, a significant three-way interaction between trial, gender and father's waist was found ( $F_{2,51} = 3.72$ ,  $p = .031$ ). Analyses investigating gender separately revealed a significant trial by father's waist interaction for female cortisol responses only ( $F_{2,35} = 4.28$ ,  $p = .022$ ). Female participants with the largest cortisol response had fathers with larger waist circumferences, and females with

the smallest cortisol response had fathers with the smallest waist circumference, when adjusting for student's own baseline cortisol levels, smoking status and waist circumference (large waist = 2.3 nmol/l; medium waist = .33 nmol/l; small waist = .06 nmol/l) (Figure 4.6). No main effect, trial by father's BMI or three-way interaction for cortisol response was found.

#### ***4.3.8.6 Interleukin-6 Reactivity and Parental Adiposity***

No interactions or main effect were found for mother's BMI or mother's waist measurement. Similarly, no main effect or interactions of father's BMI were discovered for IL-6. No interactions were discovered for father's waist circumference and IL-6. A main effect of father's waist measurement was present ( $F_{2,51} = 3.57$ ,  $p = .035$ ), although, estimated means indicate that this effect was not linear (large waist = .95 pg/ml; medium waist = .96 pg/ml; small waist = .82 pg/ml). Univariate ANCOVA revealed no difference between parental adiposity and IL-6 concentrations in relation to the tasks.

#### ***4.3.8.7 Summary of Parental Adiposity and Physiological Responsivity***

In summary, univariate analyses of covariance indicated that females whose fathers had a larger waist circumference had greater cortisol and systolic blood pressure elevations to the first stress task than females whose fathers had a low or medium waist measurement. In addition, analysis revealed that participants whose fathers had a larger waist circumference produced higher IL-6 concentrations throughout the study period. The analyses of student responsivity and parental adiposity, in general, revealed that physiological response patterns across the course of the experiment did differ by parental adiposity for many other cardiovascular variables (including systolic blood pressure, diastolic blood pressure, heart rate and heart rate variability), however these effects were curvilinear.

### 4.3.9 Family History of Hypertension and Physiological Responsivity

#### 4.3.9.1 Blood Pressure Responsivity and Family History of Hypertension

No main effect or trial by FH interaction was present for diastolic BP. A three-way interaction was found ( $F_{1,70} = 4.51$ ,  $p = .037$ ); however, univariate analyses of covariance revealed that diastolic BP reactivity and recovery from the mental stress tasks did not differ by family history of hypertension, when controlling for baseline levels, smoking status, and student's own adiposity.

No trial by family history of hypertension interaction for systolic blood pressure was discovered, but a three-way interaction was found ( $F_{5,360} = 3.12$ ,  $p = .036$ ). Univariate analyses of covariance also revealed that male participants with a negative FH of hypertension had a stronger reaction to task 1 (FH positive = 8.6 mmHg; FH negative = 11.1 mmHg) and task 2 (FH positive = 21.6 mmHg; FH negative = 24.6 mmHg) than those participants with a positive FH of hypertension (task 1,  $F_{1,16} = 4.64$ ,  $p = .047$ ; task 2,  $F_{1,16} = 5.64$ ,  $p = .030$ ). No difference between family history and systolic blood pressure was discovered for female participants.

#### 4.3.9.2 Heart Rate Responsivity and Family History of Hypertension

Repeated measure analyses revealed no main effect or interactions for heart rate.

#### 4.3.9.3 Heart Rate Variability, Pre-Ejection Period Responsivity and Family History of Hypertension

No main effect or trial by FH of hypertension interaction was discovered for HRV. A significant three-way interaction was discovered ( $F_{5,405} = 3.19$ ,  $p = .028$ ). When the sample was split by gender, univariate analyses of covariance revealed that

heart rate variability reactivity to, or recovery from the mental stress tasks did not differ according to family history of hypertension for either gender.

No interactions or main effects of FH of hypertension were found for PEP.

#### ***4.3.9.4 Cortisol, Interleukin-6 Responsivity and Family History of Hypertension***

No interactions or main effects of FH of hypertension were found for cortisol or interleukin-6 concentrations.

In summary, it does not appear, in this study, that cardiovascular, neuroendocrine or inflammatory responsivity could be differentiated by family risk of hypertension.

### **4.3.10 Family History of Cardiovascular Disease**

In addition to parental adiposity and FH of hypertension, data were also collected on student's family history of cardiovascular disease. Preliminary analyses revealed that student's BMI and WHR did not differ by family history and that subjective task ratings were the same for participants with a positive and with a negative family history of cardiovascular disease (Table 4.7). Task impact ratings were therefore not deemed to mediate any possible relationship between reactivity and family history risk so were not entered as covariates in the analyses.

#### ***4.3.10.1 Blood Pressure Responsivity and Family History of Cardiovascular Disease***

No main effect, trial by FH of cardiovascular disease or three-way interactions were present for systolic blood pressure. A marginal family history by gender interaction was discovered ( $F_{1,72} = 3.87$ ,  $p = .053$ ). When the sample was split by gender, analyses revealed that this main effect was present only in females ( $F_{1,52} = 7.43$ ,  $p = .009$ ). Means indicated that females with a positive FH of cardiovascular disease

had higher systolic blood pressure throughout the study in comparison to females with a negative family history of cardiovascular disease (positive FH of CVD = 120.1 mmHg; negative FH of CVD = 108.2 mmHg). Univariate analysis of covariance focussing specifically on systolic blood pressure task reactivity revealed no difference in task reactivity between participants with a positive and negative family history of cardiovascular disease (Table 4.8). Although, when the sample was split by gender a marginal relationship between positive family history and reactivity to the first task was suggested for female participants ( $F_{1,49} = 3.19$ ,  $p = .081$ ). A main effect of family history was also found for task recovery, indicating that participants with a positive family history of cardiovascular disease had poorer recovery from the mental stress tasks. This effect was strengthened when baseline systolic blood pressure, smoking status, gender and student's own waist to hip ratio and body mass index were accounted for ( $F_{1,70} = 4.42$ ,  $p = .039$ ). As can be seen in Table 4.8, those with a negative family history of cardiovascular disease had a mean systolic BP recovery change score of -3.11 ( $\pm 12.6$ ), indicating a systolic blood pressure level that was lower than baseline post-task. In comparison, participants with a positive family history of cardiovascular disease had a mean systolic blood pressure change score of 3.36 ( $\pm 11.1$ ), indicating that systolic blood pressure in this high risk group did not return to baseline 45 minutes post-task.

In order to examine whether both task reactivity and task recovery were independently related to family history, univariate ANCOVA was performed with systolic BP recovery as the dependent variable, family history of cardiovascular disease as the between subject factor and systolic BP during the second task as a covariate (in addition to student's own WHR, BMI, baseline systolic BP, gender and smoking status). With both factors entered into the univariate model, student's systolic BP recovery was

significantly better among participants with a negative family history of cardiovascular disease ( $F_{1,69} = 6.04$ ,  $p = .017$ ), indicating that the effect of recovery was independent of one's response to the second stress test.

Repeated measures ANOVA revealed a significant trial by family history interaction for diastolic blood pressure ( $F_{1,70} = 13.56$ ,  $p = .001$ ) (Figure 4.7). No main effect or three-way interaction was present. Univariate analysis of covariance indicated that participants with a positive family history of cardiovascular disease had a significantly stronger diastolic BP reaction to the first stress task ( $F_{1,68} = 7.76$ ,  $p = .007$ ) and significantly poorer recovery ( $F_{1,68} = 7.06$ ,  $p = .01$ ) when controlling for students' own baseline diastolic BP, smoking status, WHR and BMI. Table 4.8, shows that participants with a negative family history of cardiovascular disease had a mean diastolic BP recovery change score of  $-2.22$  mmHg ( $\pm 7.9$ ), indicating that diastolic blood pressure was lower than baseline post-task. In comparison, participants with a positive family history of cardiovascular disease had a mean diastolic blood pressure change score of  $3.85$  mmHg ( $\pm 8.4$ ), indicating that diastolic blood pressure in this high risk group did not return to baseline 45 minutes post-task. Again, diastolic BP recovery values and diastolic BP response to the second task were both entered into the univariate model to examine whether these effects were independently related to family history of cardiovascular disease. When diastolic BP response to the second task was controlled for, recovery from the mental tasks was still related to FH of cardiovascular disease ( $F_{1,67} = 8.04$ ,  $p = .006$ ). This indicated that participants with a positive FH of cardiovascular disease had greater diastolic blood pressure reactivity to the first task and poorer recovery from the stress tasks.



#### ***4.3.10.2 Heart Rate Responsivity and Family History of Cardiovascular Disease***

No main effect, trial by FH of cardiovascular disease or three-way interactions was present for heart rate. However, univariate analysis of covariance did show a significant gender by family history interaction of heart rate reactivity for the first task ( $F_{1,83} = 5.69, p = .019$ ) when controlling for baseline heart rate and student's own BMI and WHR (Table 4.8). The sample was then split by gender and analyses revealed that female participants with a positive history of cardiovascular disease were more reactive to the first stress task ( $F_{1,54} = 8.74, p = .005$ ) than female participants with a negative family history, when controlling for covariates (positive FH of CVD mean change score, 8.4 bpm; negative FH of CVD mean change score 2.7 bpm). No difference was found for male participants. No difference was found for heart rate recovery or reactivity to task 2 for those with a positive and negative family history of cardiovascular disease.

#### ***4.3.10.3 Heart Rate Variability Responsivity and Family History of Cardiovascular Disease***

No main effects, trial by FH of cardiovascular disease or three-way interactions were present for heart rate variability. However, univariate analysis of covariance did show a significant gender by family history interaction of heart rate variability reactivity for the first task ( $F_{1,31} = 4.48, p = .026$ ) when controlling for baseline heart rate and student's own BMI and WHR. When the sample was split by gender, female participants with a positive family history were marginally more reactive to task 1 ( $F_{1,19} = 3.37, p = .082$ ) and significantly more reactive to task 2 ( $F_{1,19} = 7.52, p = .013$ ) than female participants with a negative risk of cardiovascular disease (Task 2 FH positive mean change scores = -22.7; FH negative mean change scores = -4.0); male participants did not differ in terms of HRV and family history risk. No differences in heart rate

recovery and reactivity to task 1 were found for those with a positive and negative family history of cardiovascular disease (Table 4.8).

#### ***4.3.10.4 Pre-Ejection Period Responsivity and Family History of Cardiovascular Disease***

No main effect, three-way or trial and family history interaction was found for pre-ejection period. Univariate ANCOVA also indicated that participants' PEP reactivity to, and recovery from the tasks did not differ by family history of cardiovascular disease (Table 4.8).

#### ***4.3.10.5 Cortisol Reactivity and Family History of Cardiovascular Disease***

No main effect, three-way or trial by FH of cardiovascular disease interaction was found for cortisol. Univariate analysis of covariance showed that individuals with a positive family history of cardiovascular disease did not differ in cortisol reactivity from those with no family history of cardiovascular (Table 4.8).

#### ***4.3.10.6 Interleukin-6 Responsivity and Family History of Cardiovascular Disease***

Interleukin-6 concentrations taken during the four experimental time points were compared for those with a positive and those with a negative FH of cardiovascular disease. No main effect, three-way or trial by FH of cardiovascular disease interaction was found for IL-6.

For exploratory reasons, risk of cardiovascular disease was reclassified from negative versus positive family history risk, to low or moderate versus strong family history risk of cardiovascular disease. Participants with a low or moderate FH of cardiovascular disease were those with one or no parents with at least one cardiovascular disease-related condition, or two grandparents (or less), diagnosed with at least one cardiovascular condition. In comparison, participants with a strong family

history of cardiovascular disease were those with more than one parent, or more than 2 grandparents with at least one cardiovascular disease-related condition. When the repeated measures analysis was rerun, a main effect of FH of cardiovascular disease was found ( $F_{1,77} = 8.63$ ,  $p = .004$ ). This indicated that participants with a moderate family history risk had significantly lower IL-6 concentrations throughout the experimental procedure (moderate FH of CVD = .82 pg/ml; strong FH of CVD = .96 pg/mm) (Figure 4.8). Mean change scores were then calculated by subtracting IL-6 concentrations at baseline from IL-6 concentrations 45 minutes post-task. Univariate analysis of covariance, controlling for student's own baseline IL-6 concentration, smoking status, BMI and WHR revealed a significant main effect of family history of cardiovascular disease ( $F_{1,74} = 7.20$ ,  $p = .009$ ), indicating that participants with a strong family history of cardiovascular disease had greater IL-6 change scores to the stress tasks 45 minutes post-task, in comparison to those with a moderate family history of cardiovascular disease (moderate FH of CVD mean IL-6 change score = .15 pg/ml; strong FH of CVD mean IL-6 change score = .36 pg/mm) (Table 4.8).

#### ***4.3.10.7 Summary of Family History of Cardiovascular Disease and Physiological Responsivity***

Analysis of family history of cardiovascular disease indicated that participants with a positive family history did differ in cardiovascular and cytokine responsivity during the experimental time points. Further analyses concentrating on participants' reactivity to the mental stress tasks revealed that those with a positive family history of cardiovascular disease had a significantly larger diastolic BP reaction to the first task than those participants with no family history risk. Heart rate and heart rate variability reactivity to tasks were elevated in women with a positive family history. IL-6 responsivity was also greater, but only when using a slightly less sensitive family

history classification. In addition, participants with a positive family history of cardiovascular disease had worse systolic and diastolic BP recovery than participants with no risk of cardiovascular disease. No effect of family history was found for cortisol reactivity.

**Table 4.2: Student Sample Characteristics (unadjusted means  $\pm$  sd)**

	<b>Total Sample (n = 103)</b>	<b>Male (n = 34)</b>	<b>Female (n = 69)</b>
Age	21.40 $\pm$ 2.1	21.53 $\pm$ 2.16	21.33 $\pm$ 2.06
Waist to hip ratio	.781 $\pm$ .117	.847 $\pm$ .077	.749 $\pm$ .119
Body Mass Index (kg/m <sup>2</sup> )	23.36 $\pm$ 3.54	23.38 $\pm$ 2.38	23.35 $\pm$ 4.01
Exercise Level (hours per 4 wks)	31.71 $\pm$ 26.0	26.88 $\pm$ 22.82	27.64 $\pm$ 23.4
Alcohol Consumption (units per day)	2.84 $\pm$ 1.1	3.1 $\pm$ 1.17	2.69 $\pm$ 1.05
Cigarette Smokers (%)	16 (15.5 %)	4 (11.8 %)	12 (17.4 %)
Diastolic Blood Pressure (mmHg)	65.27 $\pm$ 8.3	65.66 $\pm$ 6.9	65.11 $\pm$ 8.8
Systolic Blood Pressure (mmHg)	114.69 $\pm$ 11.7	122.95 $\pm$ 11.9	111.55 $\pm$ 10.0
Heart Rate (bpm)	71.18 $\pm$ 9.4	69.45 $\pm$ 11.0	72.04 $\pm$ 8.8
Heart Rate Variability (ms)	56.47 $\pm$ 30.0	57.28 $\pm$ 33.1	59.92 $\pm$ 42.5
Pre-Ejection Period (ms)	123.63 $\pm$ 9.1	124.6 $\pm$ 10.0	123.18 $\pm$ 8.7
Cortisol (nmol/l)	5.79 $\pm$ 3.2	6.04 $\pm$ 3.6	5.68 $\pm$ 3.0
Interleukin-6 (pg/ml)	.72 $\pm$ .41	.77 $\pm$ .52	.70 $\pm$ .36

**Table 4.3: Parent Sample Characteristics (unadjusted means  $\pm$  sd)**

	<b>Parental (n = 158)</b>	<b>Mother (n = 87)</b>	<b>Father (n = 71)</b>
Age	52.8 $\pm$ 5.3	51.5 $\pm$ 4.7	54.4 $\pm$ 5.5
Body Mass Index (kg/m <sup>2</sup> )	25.70 $\pm$ 4.2	25.12 $\pm$ 4.1	26.44 $\pm$ 4.3
Waist Measurement (cm)	84.10 $\pm$ 16.2	77.73 $\pm$ 13.8	91.02 $\pm$ 15.8
Cigarette Smokers (%)	20 (12.7 %)	7 (8.0 %)	13 (18.3 %)
High Cholesterol (%)	33 (20.9 %)	13 (14.9 %)	20 (28.6 %)
High Blood Pressure (%)	31 (19.6 %)	17 (19.5 %)	14 (20.0 %)
Heart Disease (%)	5 (3.2 %)	1 (1.1 %)	4 (5.6 %)
Diabetes (%)	10 (6.3 %)	3 (3.4 %)	7 (9.9 %)
Total Incidence (%)	79 (50.0 %)	34 (39.1 %)	45 (63.4 %)

Table 4.4: Cardiovascular, Neuroendocrine and Cytokine Responses to the Experimental Procedure (unadjusted means  $\pm$  sd)

	Baseline	Task 1 (Stroop)	Task 2 (Speech)	15 min post- task	30 min post- task	45 min post- task	90 min post- task
Subjective Stress Ratings	2.04 $\pm$ 1.1	4.56 $\pm$ 1.2	4.27 $\pm$ 1.4	1.88 $\pm$ .72	1.59 $\pm$ .66	1.64 $\pm$ .80	1.93 $\pm$ 1.3
Diastolic BP (mmHg) male	65.66 $\pm$ 6.9	78.37 $\pm$ 11.1	85.05 $\pm$ 11.1	73.37 $\pm$ 8.7	73.15 $\pm$ 8.3	71.01 $\pm$ 9.3	
female	65.11 $\pm$ 8.8	72.32 $\pm$ 12.7	78.67 $\pm$ 12.9	71.30 $\pm$ 12.1	69.65 $\pm$ 11.3	67.11 $\pm$ 10.8	
Systolic BP (mmHg) male	122.95 $\pm$ 11.9	141.71 $\pm$ 24.5	151.68 $\pm$ 28.2	133.65 $\pm$ 14.1	131.30 $\pm$ 14.0	128.67 $\pm$ 15.7	
female	111.55 $\pm$ 10.0	118.94 $\pm$ 16.7	129.68 $\pm$ 21.4	118.61 $\pm$ 13.6	116.40 $\pm$ 14.0	112.39 $\pm$ 14.1	
Heart Rate (bpm)	71.18 $\pm$ 9.4	78.53 $\pm$ 11.9	86.85 $\pm$ 14.6	69.88 $\pm$ 9.6	69.59 $\pm$ 9.4	69.6 $\pm$ 9.4	
Heart Rate Variability (ms)	56.47 $\pm$ 30.0	41.44 $\pm$ 21.7	36.87 $\pm$ 18.1	54.9 $\pm$ 27.7	57.12 $\pm$ 30.8	56.76 $\pm$ 29.3	
Pre-Ejection Period (ms)	123.63 $\pm$ 9.1	119.55 $\pm$ 10.2	113.52 $\pm$ 12.3	125.09 $\pm$ 8.8	125.57 $\pm$ 8.8	126.25 $\pm$ 9.0	
Interleukin-6 (pg/ml) male	.77 $\pm$ .52		.79 $\pm$ .52			.79 $\pm$ .37	.97 $\pm$ .52
female	.70 $\pm$ .36		.78 $\pm$ .38			.99 $\pm$ .57	1.49 $\pm$ 1.2
Cortisol (nmol/l)	5.79 $\pm$ 3.2		<i>Task Maximum</i> 7.15 $\pm$ 5.1				

**Table 4.5: Subjective Appraisals of Tasks (unadjusted means  $\pm$  sd)**

	<b>Task 1</b>	<b>Task 2</b>
Perceived Performance	2.17 $\pm$ 1.7	4.22 $\pm$ 1.5
Task Difficulty	5.48 $\pm$ 1.9	3.64 $\pm$ 1.7
Task Involvement	4.91 $\pm$ 1.9	5.72 $\pm$ 1.4
Task Controllability	4.78 $\pm$ 1.7	2.94 $\pm$ 1.9

All ratings on 7 point scales where 1 = low and 7 = high



Table 4.6: Mean Cardiovascular, Neuroendocrine and Cytokine Responsivity to Tasks 1 and 2 (unadjusted means ± sd)

	Male	Female	Complete Sample	Range	
Diastolic BP (mmHg)	Task 1	12.72 ± 8.3	7.21 ± 8.5	8.77 ± 8.8	-12.07 to 32.95
	Task 2	19.39 ± 8.6	13.56 ± 8.8	15.21 ± 9.1	-1.05 to 41.32
	Recovery	5.36 ± 6.4	2.0 ± 8.6	2.95 ± 8.1	-15.66 to 20.14
Systolic BP (mmHg)	Task 1	18.76 ± 17.6	7.40 ± 11.5	10.53 ± 14.3	-17.78 to 65.02
	Task 2	36.73 ± 22.4	18.14 ± 16.4	23.7 ± 20.0	-10.03 to 86.69
	Recovery	5.71 ± 11.6	.84 ± 10.96	2.18 ± 11.3	-24.72 to 44.06
Heart Rate (bpm)	Task 1	6.55 ± 7.95	7.74 ± 6.3	7.35 ± 6.9	-9.37 to 30.49
	Task 2	16.45 ± 11.1	15.27 ± 11.2	15.66 ± 11.1	-2.41 to 49.39
	Recovery	-2.36 ± 5.0	-1.20 ± 4.1	-1.58 ± 4.5	-17.83 to 14.88
Heart Rate Variability (ms)	Task 1	-16.13 ± 20.1	-16.91 ± 16.9	-16.13 ± 17.9	-86.04 to 12.83
	Task 2	-24.36 ± 18.1	-18.77 ± 16.2	-20.68 ± 16.9	-55.73 to 16.42
	Recovery	2.80 ± 19.1	-1.20 ± 12.2	.034 ± 14.7	-47.45 to 39.84
Pre-Ejection Period (ms)	Task 1	-5.85 ± 7.4	-3.27 ± 6.2	-4.08 ± 6.7	-25.0 to 21.34
	Task 2	-12.65 ± 8.9	-8.71 ± 10.8	-9.9 ± 10.4	-54.72 to 6.67
	Recovery	2.97 ± 5.3	2.68 ± 5.2	2.76 ± 5.2	-21.78 to 18.22
Cortisol (nmol/l)	Tasks 1 & 2	2.05 ± 3.8	1.07 ± 3.4	1.36 ± 6.5	1.84 to 36.58
Interleukin-6 (pg/ml)	Tasks 1 & 2	.22 ± .54	.79 ± 1.2	.62 ± 1.1	-1.55 to 5.28

**Table 4.7: Student Adiposity and Task Ratings by Family History of Cardiovascular Disease (unadjusted means  $\pm$  sd)**

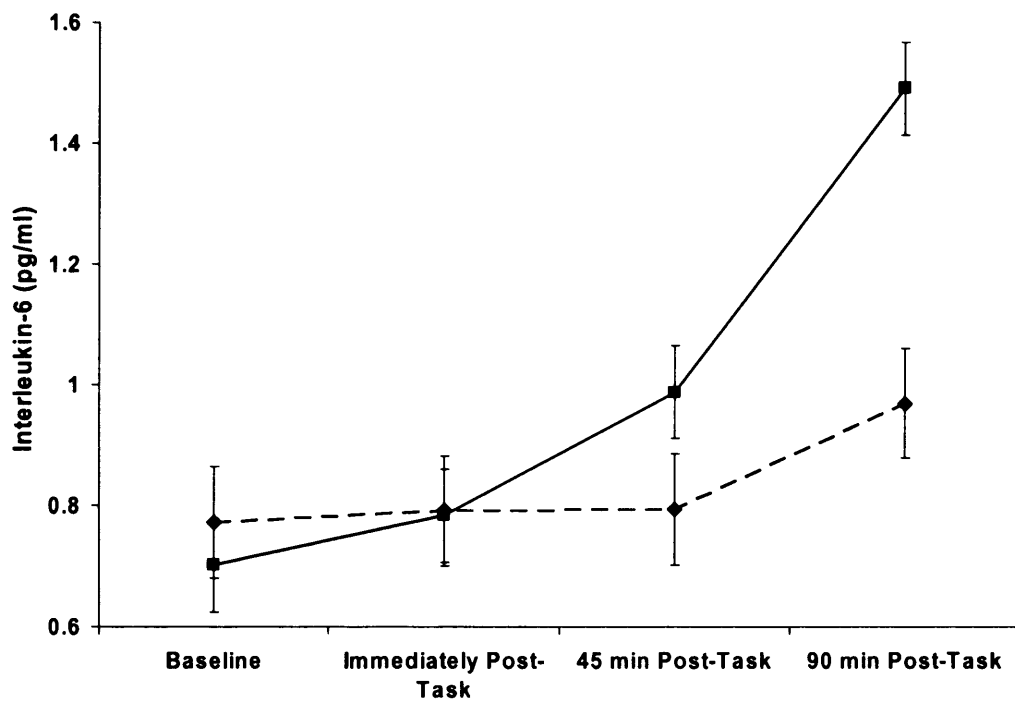
		Negative Family History	Positive Family History
Body Mass Index (kg/m <sup>2</sup> )		23.23 $\pm$ 3.6	23.57 $\pm$ 3.69
Waist to hip ratio		.750 $\pm$ .097	.790 $\pm$ .119
Perceived Performance	Task 1	2.25 $\pm$ .93	2.36 $\pm$ .95
	Task 2	4.31 $\pm$ 1.2	4.0 $\pm$ 1.3
Task Difficulty	Task 1	5.19 $\pm$ 1.5	5.48 $\pm$ .99
	Task 2	3.88 $\pm$ 2.1	4.11 $\pm$ 1.7
Task Involvement	Task 1	5.31 $\pm$ 1.3	4.87 $\pm$ 1.3
	Task 2	5.12 $\pm$ 1.1	5.08 $\pm$ 1.1
Task Controllability	Task 1	2.81 $\pm$ .83	2.57 $\pm$ 1.0
	Task 2	4.31 $\pm$ 1.5	4.40 $\pm$ 1.5
Subjective Stress Rating	Task 1	4.38 $\pm$ 1.1	4.67 $\pm$ 1.2
	Task 2	4.13 $\pm$ 1.6	4.28 $\pm$ 1.4
Relaxation Rating	Task 1	2.81 $\pm$ 1.1	2.84 $\pm$ 1.2
	Task 2	3.31 $\pm$ 1.8	3.27 $\pm$ 1.3

All ratings on 7 point scales where 1 = low and 7 = high

**Table 4.8: Cardiovascular, Neuroendocrine and Cytokine Baseline and Responsivity levels by Family History of Cardiovascular Disease (unadjusted means  $\pm$  sd)**

		Negative Family History	Positive Family History
Diastolic BP (mmHg)	Baseline	63.84 $\pm$ 9.1	65.67 $\pm$ 8.3
	Task 1	2.58 $\pm$ 9.6	10.23 $\pm$ 8.1
	Task 2	15.0 $\pm$ 11.6	15.85 $\pm$ 8.67
	Recovery	-2.22 $\pm$ 7.9	3.85 $\pm$ 8.4
Systolic BP (mmHg)	Baseline	110.51 $\pm$ 13.2	115.25 $\pm$ 11.2
	Task 1	5.36 $\pm$ 18.1	11.40 $\pm$ 12.9
	Task 2	22.22 $\pm$ 30.56	24.0 $\pm$ 17.4
	Recovery	-3.11 $\pm$ 12.6	3.36 $\pm$ 11.1
Heart Rate (bpm)	Baseline	69.81 $\pm$ 10.2	71.27 $\pm$ 9.4
	Task 1	5.26 $\pm$ 6.3	7.37 $\pm$ 6.8
	Task 2	16.05 $\pm$ 12.7	15.74 $\pm$ 11.3
	Recovery	-1.84 $\pm$ 3.81	-1.64 $\pm$ 4.7
Heart Rate Variability (ms)	Baseline	52.78 $\pm$ 26.9	59.18 $\pm$ 31.6
	Task 1	-8.86 $\pm$ 16.5	-18.71 $\pm$ 19.0
	Task 2	-12.71 $\pm$ 18.2	-23.48 $\pm$ 16.9
	Recovery	2.40 $\pm$ 11.7	.16 $\pm$ 15.9
Pre-Ejection Period (ms)	Baseline	124.01 $\pm$ 8.17	124.39 $\pm$ 9.4
	Task 1	-3.30 $\pm$ 5.70	-3.55 $\pm$ 6.6
	Task 2	-11.27 $\pm$ 14.28	-9.66 $\pm$ 9.3
	Recovery	3.73 $\pm$ 4.8	2.24 $\pm$ 5.0
Cortisol (nmol/l)	Baseline	9.44 $\pm$ 6.8	8.48 $\pm$ 3.8
	Tasks 1 & 2	.38 $\pm$ .50	.22 $\pm$ .57
Interleukin-6 (pg/ml)	Baseline	.69 $\pm$ .33	.72 $\pm$ .35
	Tasks 1 & 2	.55 $\pm$ 1.1	.62 $\pm$ 1.1
	Baseline*	.66 $\pm$ .32	.76 $\pm$ .36
	Tasks 1 & 2*	.38 $\pm$ .77	.78 $\pm$ 1.2

\* Family history reclassification



**Figure 4.2: Male (dashed line) and Female (solid line) Mean Interleukin-6 Concentration over the Study Period. Mean values are adjusted for participant's own baseline IL-6 concentrations, body mass index, waist to hip ratio and smoking status. Error bars are standard error of the mean.**

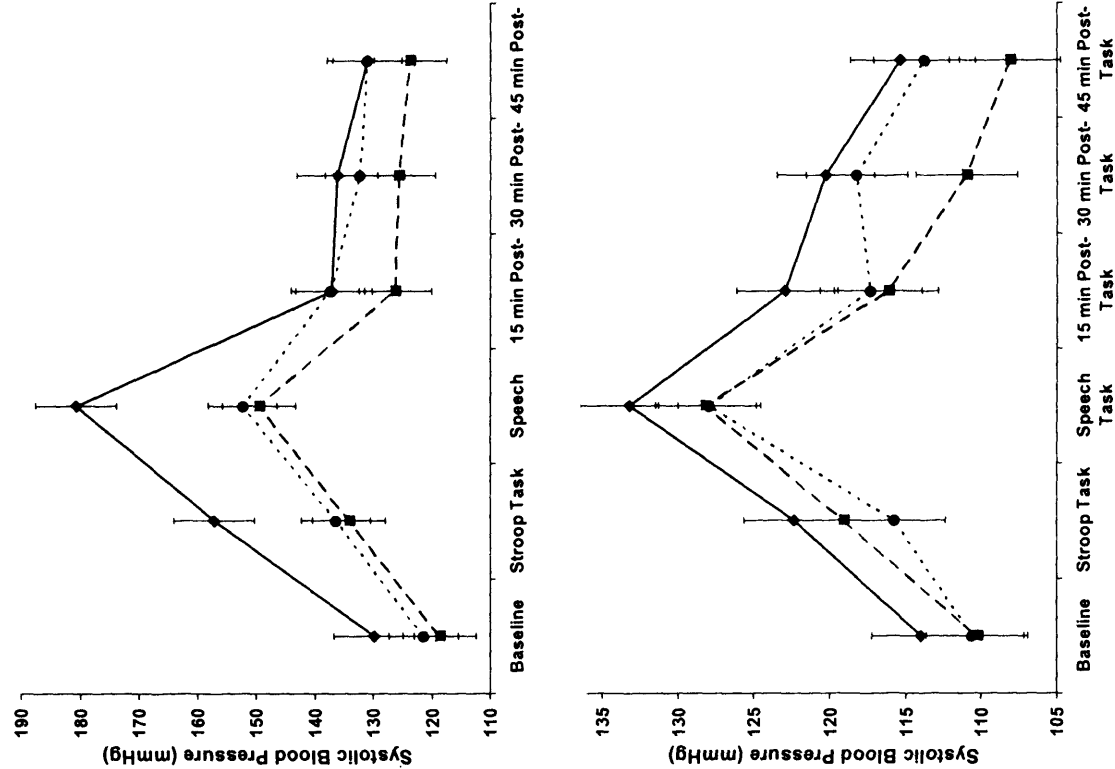


Figure 4.3: Male (upper panel) and Female (lower panel) Mean Systolic Blood Pressure over the Study Period for Participants with a High (solid line), Medium (dashed line) and Low (dotted line) Waist to Hip Ratio. Mean values are adjusted for participant's own body mass index, and smoking status. Error bars are standard error of the mean.

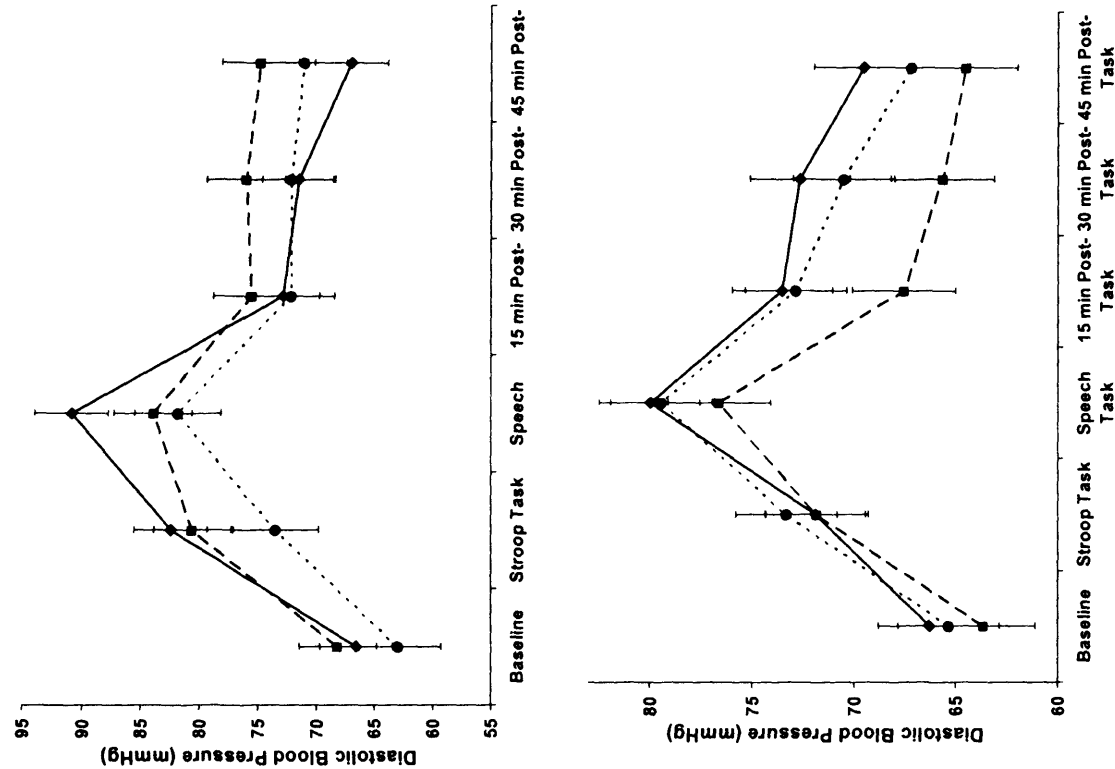
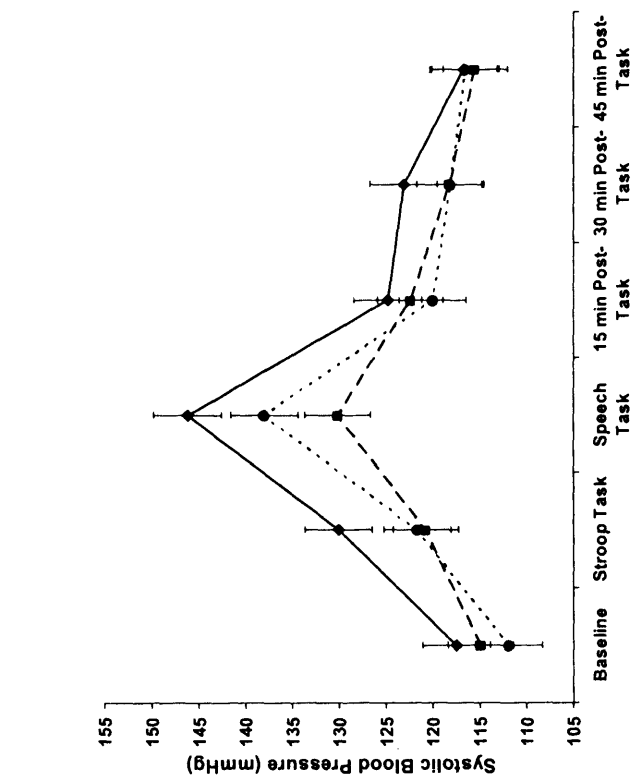
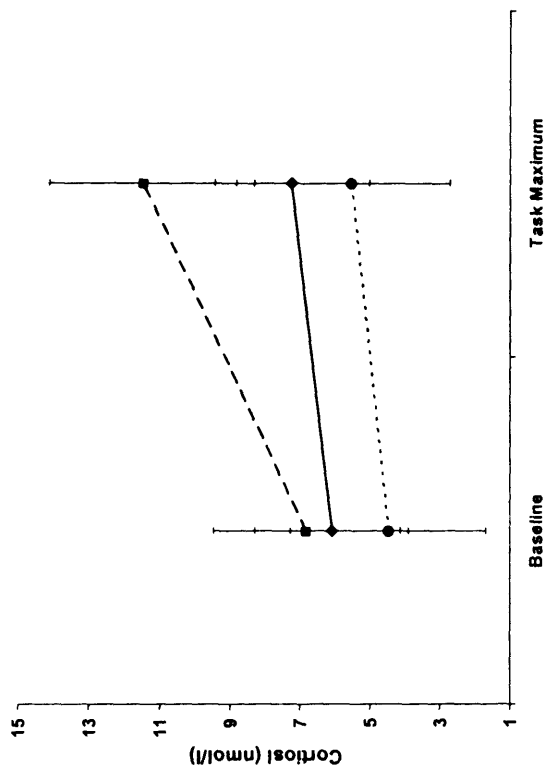
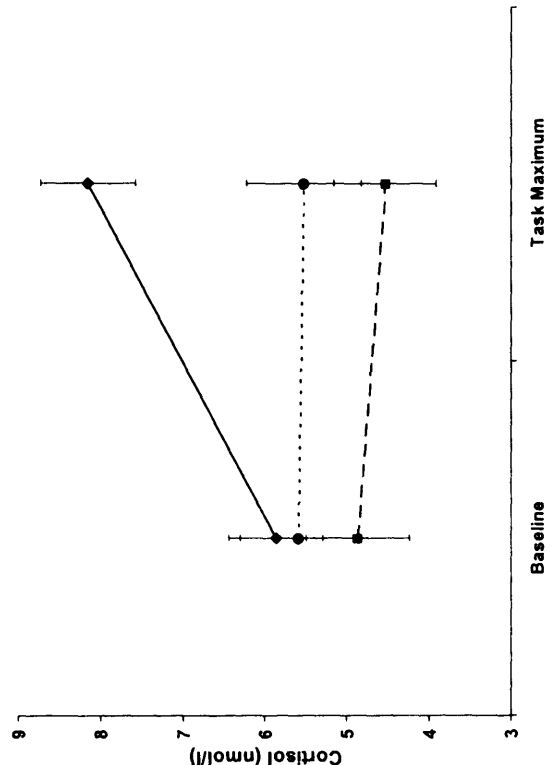


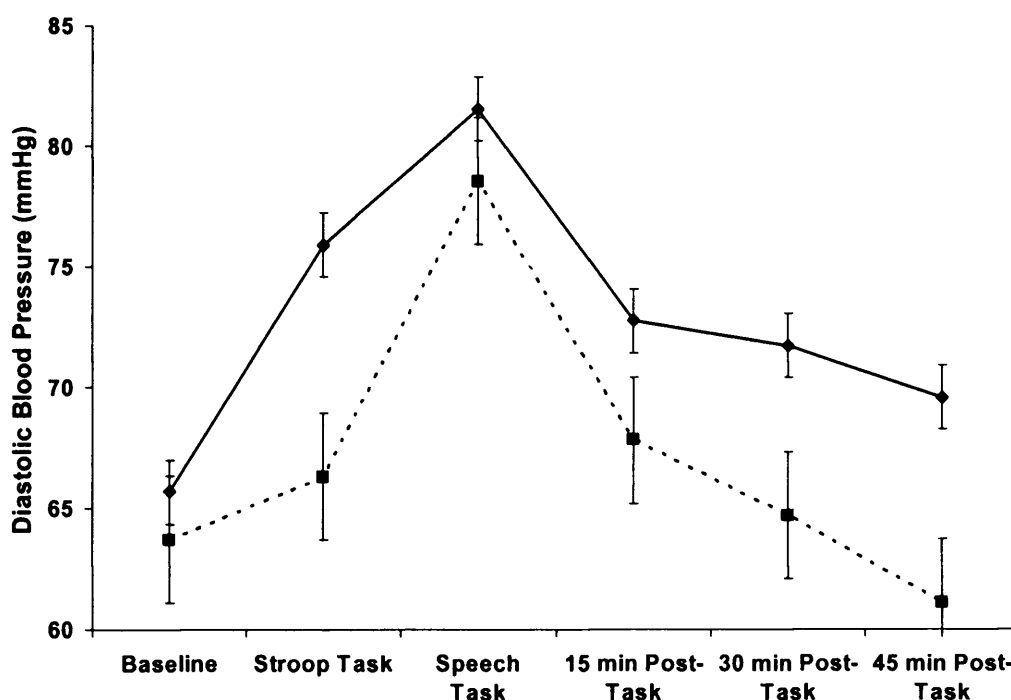
Figure 4.4: Male (upper panel) and Female (lower panel) Mean Diastolic Blood Pressure over the Study Period for Participants with a High (solid line), Medium (dashed line) and Low (dotted line) Waist to Hip Ratio. Mean values are adjusted for participant's own body mass index, and smoking status. Error bars are standard error of the mean.



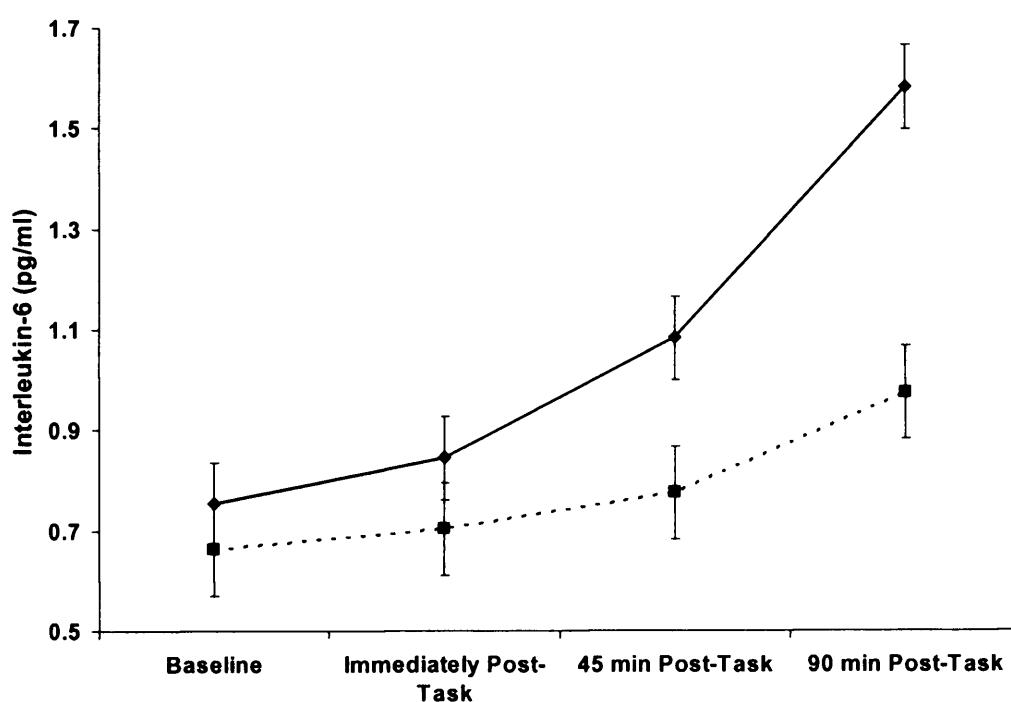
**Figure 4.5: Mean Systolic Blood Pressure over the Study Period for Participants whose Fathers had a High (solid line), Medium (dashed line) or Low (dotted line) Body Mass Index. Mean values are adjusted for participant's own baseline systolic blood pressure, body mass index, gender and smoking status. Error bars are standard error of the mean.**



**Figure 4.6: Male (upper panel) and Female (lower panel) Mean Cortisol Concentrations over the Study Period for Participants whose Father had a High (solid line), Medium (dashed line) or Low (dotted line) Waist to Hip Ratio. Mean values are adjusted for participant's own body mass index, and smoking status. Error bars are standard error of the mean.**



**Figure 4.7: Mean Diastolic Blood Pressure over the Study Period for Participants with a positive (solid line) and Negative (dashed line) Family History of Cardiovascular Disease. Mean values are adjusted for participant's own baseline Diastolic Blood Pressure, body mass index, waist to hip ratio, gender and smoking status. Error bars are standard error of the mean.**



**Figure 4.8: Mean Interleukin-6 Concentrations over the Study Period for those with a Strong (solid line) and low or Moderate (dashed line) Family History of Cardiovascular Disease. Mean values are adjusted for participant's own baseline IL-6 concentrations, body mass index, waist to hip ratio, gender and smoking status. Error bars are standard error of the mean.**

## 4.4 Discussion

The association between disturbances in physiological responsivity and cardiovascular disease is well established (Steptoe & Tavazzi, 1996; Steptoe, 1997); however, the role of hyper-responsivity in the development of this disorder is less clear. Identifying the contribution of physiological responsivity to the relationship between stress and health can be achieved by examining family history and physiological stress responsivity of young adults who have yet to develop the disease. On this principle it was hypothesised that participants with a positive family history of cardiovascular disease would exhibit greater physiological responses and impaired recovery to two laboratory-based mental stress tasks.

In summary, five major findings merit discussion. First, the two mental stress tasks produced marked physiological changes over the course of the experiment. Second, a positive family history of hypertension did not appear to be associated with exaggerated cardiovascular, neuroendocrine or cytokine response in young adults. Third, a positive family history of cardiovascular disease was associated with greater diastolic blood pressure, heart rate, heart rate variability and interleukin-6 responses to the stressors, while systolic and diastolic blood pressure recovery were related to a positive family history of cardiovascular disease. The results also suggest that these relationships were independent of gender, and student's weight and smoking status. Fourth, cardiovascular, and neuroendocrine stress responses were related to parental adiposity when obesity was considered as a separate independent risk factor for cardiovascular disease. Lastly, student's appraisals of the two tasks did not differ by family history or obesity risk.



#### **4.4.1 Physiological and Psychological Responsivity to the Mental Stress Tasks**

Two mental stress tasks were administered during the experimental procedure; a colour-word interference Stroop task and a public speaking challenge. The combination of these two tasks is recommended because of the levels of physiological responsivity, social-evaluative threat and feeling of uncontrollability they produce (Dickerson & Kemeny, 2004). Results revealed that the tasks induced feelings of stress, difficulty, poor performance and uncontrollability. Importantly, significant increases in cardiovascular, neuroendocrine and cytokine production were also found. In addition, men had greater systolic and diastolic blood pressure reactivity to the tasks and poorer recovery from the challenges than women, even though female participants tended to rate the tasks as more stressful than males. This gender difference supports the findings of previous research and has potentially important implications for hypotheses regarding the role of reactivity in determining risk for cardiovascular disease (Stoney et al, 1988). Evidence, including the results of this study, implies that exaggerated cardiovascular stress responses may be related to an increased risk of cardiovascular disease. The analysis of gender differences in reaction to stress may help explain why today, females in most industrialised countries are protected from cardiovascular disease relative to males.

Kudielka et al (1998) also report that males tend to exhibit greater cortisol responses to stress; however, results from the current study failed to replicate this finding. This was largely because only a small increase in cortisol occurred following the stressor. For this present sample the increase was just 10 %. In contrast, previous studies similar to the current investigation, reporting a positive relationship between adiposity and cortisol reactivity have demonstrated neuroendocrine increases of

approximately 92 % (Epel et al, 2000) and 33 % (Marin et al, 1992). Although Dickerson and Kemeny (2004) report that ‘active stressors’ which induce feelings of social threat tend to produce the highest endocrine change scores, the speech task employed in this study generated only a weak increase in cortisol concentration. It is possible therefore, that the two stressors administered were not sufficiently intense to elicit adequate cortisol responses or did not constitute a sufficient social threat to the sample. This may be explained by the fact student samples are relatively familiar with performing tasks under stressful situations in a controlled environment. Alternatively, it is possible that cortisol may have already risen in response to cannulation, since blood sampling is a potential stressor for many individuals (causing a small percentage of the sample in this study to faint). This modest increase in cortisol may consequently explain the null results observed for cortisol reactivity among participants with increased weight or a positive family history of hypertension and cardiovascular disease.

Crucial between-task differences were also discovered. It was revealed that all cardiovascular variables, except heart rate variability, were significantly elevated during the second task (cortisol and IL-6 task responses were not analysed separately for each task). Subsequently, it was shown that reactivity to the second task was not related to any family history differences. These null findings suggest that either tasks which assess more ecologically valid situations are not related to family history differences, or more probably, the extremely high responses for the second task produced a ceiling effect. This ceiling effect suggests that inter-individual differences between participants with a positive and negative family history may have been obscured as all participants found this task to be particularly stressful. This ceiling effect may also be an accumulation of stress response carried over from task 1, so the effect may not be due

solely to responsivity to the second task. It should also be noted that significant differences between family history and heart rate variability at task 2 were found, and that no ceiling effect was observed for heart rate variability during this task. This further suggests that the failure to find family history differences between the other cardiovascular variables may be explained by lack of variability in stress response to the second task.

#### **4.4.2 Family History of Hypertension and Physiological Stress**

##### **Responsivity**

A large and complex literature has attempted to determine whether cardiovascular responsivity precedes hypertension onset by investigating normotensive individuals with differing degrees of family history risk. A review of literature published in the 1970s and 1980s reported that only 35 % of studies showed an association between systolic blood pressure, diastolic blood pressure or heart rate reactivity and family history risk of hypertension (Manuck et al, 1993). A mini-review of the literature published since 1990, conducted specifically for this study, revealed that the relationship between cardiovascular responsivity and family history of hypertension is still inconclusive (see Table 4.1). This inconsistency is often attributed to methodological limitations. In order to replicate and extend previous research, the present study investigated cardiovascular, neuroendocrine and cytokine responses to ‘active psychological stressors’ in a large sample of young normotensive participants who had a family history of hypertension across two generations.

Despite methodological improvements, the investigation found no evidence of cardiovascular, neuroendocrine or cortisol responsivity differences between subjects with a family history of hypertension and those without. This is in accordance with the

finding of several studies who reported null results (Perini et al, 1988; Perini et al, 1990; Ravogli et al, 1990; Gerin & Pickering, 1995). However, due to certain characteristics of the current sample, it would be erroneous to conclude that a family history design is not effective when trying to determine the relationship between physiological responsivity and hypertension.

As a result of the descriptive nature of the 'quasi-experiment', equal group sizes could not be pre-selected. The sample was, therefore, assigned to family history positive and family history negative groups once physiological data had been collected. This resulted unfortunately, in a restricted range of hypertensive symptoms among first-degree relatives. For example, only 2 participants (1.9 %) reported both parents with high blood pressure; while only 26 participants (25.2 %) had one or more grandparents with a history of hypertension. It would have been desirable to compare groups with two hypertension positive parents with those with two normotensive parents but these sample restrictions meant that this was not possible. Restricted range of parental hypertension may be a result of the young age of the sample and therefore the young age of their parents, who may be yet to develop hypertension (parental mean age 53.1 years  $\pm$  4.5). The null results could also be attributed to the small sample size or categorisation error resulting in false negative classifications. Correct classification is central to the discussion of the family history of cardiovascular disease results in general. A more detailed discussion of these points can be found in Section 4.4.3.5.

### **4.4.3 Family History of Cardiovascular Disease and Stress**

#### **Responsivity**

The majority of studies employing a family history design tend to investigate the relationship between cardiovascular responsivity and risk of hypertension. The present

study extended this design in order to explore the relationship between cardiovascular, neuroendocrine and cytokine responsivity and risk of future development of cardiovascular disease. Previous studies have demonstrated a difference in cardiovascular reactivity for those with a positive family history of cardiovascular disease; however, cardiovascular disease was defined solely as incidence of myocardial infarction and myocardial infarction in middle-aged men and women is relatively low (Stoney & Matthews, 1988; Treiber et al, 1991; 1993). Many other conditions besides myocardial infarction comprise cardiovascular disease, including hypertension, high cholesterol, coronary heart disease and diabetes. The present study therefore classified cardiovascular disease as the combined incidence of these four conditions.

#### ***4.4.3.1 Family History of Cardiovascular Disease and Cardiovascular Reactivity***

Using a cumulative score for both parents and grandparents it was found that participants with a positive family history of cardiovascular disease had exaggerated diastolic blood pressure responses to the first mental stress task. This effect persisted when students' own waist to hip ratio, body mass index, baseline blood pressure level and smoking status were accounted for. The findings suggest that stress-related increases in diastolic blood pressure in the family history positive group were not simply due to cardiovascular disease-related factors already present in this at risk group.

Family history group differences were also examined for other cardiovascular factors, including systolic blood pressure, heart rate, heart rate variability and pre-ejection period. When the whole sample was assessed no differences in reactivity were detected. For exploratory reasons the sample was split by gender. Results of this analysis revealed that female participants with a positive family history of cardiovascular disease had stress-related elevations in heart rate, heart rate variability and systolic blood pressure (although this last result was only marginally significant).

Due to the small sample size ( $n = 22$ ) and cell size of male participants with available family history and cardiovascular data, it cannot be concluded that there is no relationship between cardiovascular reactivity and family history of cardiovascular disease among male participants. Instead, the results suggest associations that would have been present had the study only recruited female participants.

With numerous studies investigating male samples (see Table 4.1); research has called for increased investigation of female populations (Schneider et al, 2003). The results of this investigation show that female family history positive subjects have greater stress-related cardiovascular increases than females with a negative family history of cardiovascular disease. It is believed that these findings are important when investigating the relationship between cardiovascular reactivity and future risk of cardiovascular disease development. Future studies using the family history design would benefit from a much larger sample of male participants to determine whether gender is truly a moderating factor in the relationship between stress responsivity and development of cardiovascular disease.

#### ***4.4.3.2 Family History of Cardiovascular Disease and Cardiovascular Recovery***

The difference in family history risk and cardiovascular recovery from the tasks was also investigated. Previous research examining the association between family history of hypertension and cardiovascular recovery revealed that participants with a positive family history tend to demonstrate poorer cardiovascular recovery from active coping stress tasks (Falkner et al, 1979; Anderson et al, 1989; De Visser et al, 1995; Gerin & Pickering, 1995; Hocking Schuler et al, 1997; O'Brien et al, 1998; Schneider et al, 2003); while others report no differences between the two groups (Lawler et al, 1998). The results of the present study were positive. It was revealed that less effective diastolic and systolic blood pressure recovery was present in the family history positive

group, and results were all independent of participant's own baseline levels, body mass index, waist to hip ratio and smoking status. Research also suggests that recovery from emotional tasks (such as the speech task used here) is slower than recovery from cognitive tasks (e.g. the Stroop task) (Vitaliano et al, 1995). Analyses were therefore performed to examine whether group differences in blood pressure recovery were independent of blood pressure response to the second task. It was discovered that diastolic and systolic blood pressure recovery of family history positive participants was impaired, compared with those participants with a negative family history of cardiovascular disease, when responsivity to the second task was taken into account. This indicates that the association of poorer recovery in the family history positive group was independent of reactivity to the task.

The results also highlight certain methodological issues which need consideration in future research. First, it is unclear what may be happening during the recovery period to keep blood pressure raised in the family history group. It is hypothesised that interpersonal stress from an emotionally engaging task, may lead to rumination and anger in some participants and not others. These behavioural responses may then keep the blood pressure elevated for a longer period after the stressor has ended (Glynn et al, 2002). In the present study, although all participants were asked to bring some low-stress activity to keep them occupied during the recovery period, no specific distracter was given. It is unclear therefore, whether rumination in certain subjects did take place. It would be interesting to measure post-task behavioural responses in future studies to assess whether family history positive and family history negative subjects differ in terms of rumination.

Second, no group differences were found between family history risk for heart rate, heart rate variability or pre-ejection period. These three variables differ from

blood pressure measurement in the fact that they typically are much swifter to return to baseline. In a review of the stress recovery literature, Linden et al (1997) discovered that the majority of participants (even those at risk because of elevated baseline values, or positive family histories), displayed return-to-baseline levels within 1-2 minutes upon termination of the stressor. It appears that in this study of young participants, even a first recovery period 10 minutes post-task was too long to detect any cardiac changes. As a result, assessment points immediately post-task are needed in order to investigate the association between family history and certain cardiovascular variables.

#### ***4.4.3.3 Mechanisms Relating Family History and Cardiovascular Responsivity***

Previous research and the findings of the present investigation have demonstrated that heightened cardiovascular reactivity to, and recovery from, stress is associated with an increased risk of cardiovascular disease. The mechanisms relating these two factors need to be explored. A review of the twin study literature concludes that changes in blood pressure are heritable and that common environmental influences are highly improbable (Turner & Hewitt, 1992). As a result it has been proposed that individual differences in cardiovascular function may arise from variation in the genes that code elements of the adrenergic and serotonergic systems. These include aberrations in transmitter synthesis, transmitter release and reuptake, enzymatic degradation and receptor activation. All of which may result in individual genetic susceptibility to heightened cardiovascular reactivity and ultimately cardiovascular disease (Snieder et al, 2002).

First,  $\alpha$  and  $\beta$  adrenergic receptors located in the heart, vasculature, or central nervous system are involved in cardiovascular regulation by mediation of peripheral vasodilatation. The genes *ADRA* and *ADRB* code for  $\alpha$  and  $\beta$  adrenergic receptors, respectively. These genes have relatively common and well-characterised polymorphic



variability, which may be partly responsible for elevations in blood pressure reactivity in vulnerable individuals. A polymorphism in the  $\beta_2$ -adrenergic receptor gene isoform (*ADRB2*) results in the change of an arginine residue at position 16 to a glycine residue (Arg16Gly). Li et al (2001) discovered a relationship between individuals with this polymorphism and systolic and diastolic blood pressure during stress. This amino acid change was associated with significantly higher levels of both systolic and diastolic blood pressure reactivity in a sample of over 500 twins (Snieder et al, 2002). Similarly, the change of Arg389Gly in the  $\beta_1$ -adrenergic receptor gene isoform (*ADRB1*) is associated with diastolic blood pressure reactivity (McCaffery et al, 2002). Individuals possessing these polymorphisms have also been found to have increased incidence of various cardiovascular-related conditions including diabetes, high cholesterol and hypertension (Ukkola et al, 2000; Bengtsson et al, 2001). These results suggest that vasodilatory-related genetic factors may be an important mechanism relating family history risk of cardiovascular disease and heightened cardiovascular function.

Second, the serotonergic system may be important in explaining the genetic link between risk of cardiovascular disease and stress responsivity. Williams et al (2001a) and McCaffery (2003) evaluated the impact of a polymorphism in the promoter sequence of the serotonin transporter gene (*5HTTLPR*) on cardiovascular reactivity to mental stress. Individuals with one or two long *5HTTLPR* alleles showed higher levels of the major serotonin metabolite 5H1AA in the cerebrospinal fluid, as well as greater blood pressure and heart rate responses to mental stress. These findings suggest that the *5HTTLPR* polymorphism affects serotonin function in the central nervous system, which results in an increased cardiovascular response to stress.

It is plausible that those individuals in the current study with elevated cardiovascular stress reactivity may be at increased risk of cardiovascular disease due to

genetic differences in the adrenergic or serotonergic systems. However, at present, it is uncertain whether these differences derive from the brain, the cardiovascular system or sympathetic nervous system. In addition, there is evidence that genetic variation in serotonergic function is associated with psychological and behavioural characteristics known to contribute to health-damaging increases in cardiovascular function. It has been found that individuals with polymorphisms in the *5HTTLPR* genotype scored higher on personality dimensions such as, anger, hostility, depression and neuroticism (Lesch et al, 1996). It must be considered, therefore, that genetic variation in personality traits may account for individual differences in reactivity phenotype. The present investigation found no association between behavioural task ratings, stress responsivity and family risk. However, future research would need to investigate the association of other personality characteristics, whilst also examining the genetic vulnerability of the sample in order to determine precisely how the adrenergic and serotonergic systems may associate stress reactivity and risk of future cardiovascular disease.

#### ***4.4.3.4 Family History of Cardiovascular Disease, Cortisol and Interleukin-6***

##### ***Reactivity***

Relatively little work has investigated the relationship between neuroendocrine reactivity and family history of cardiovascular disease risk factors. Although there appears to be a link between increased cortisol production and cardiovascular disease-related conditions (Girod & Brotman, 2004), only one study has examined this relationship using a family history design. Fredrickson et al (1991) discovered that healthy participants with a family history of hypertension had an elevated cortisol response to stress, but only for active coping tasks. The present study attempted to extend these findings by assessing cortisol reactivity and family history of hypertension

and cardiovascular disease. Results revealed however, that cortisol reactivity in this young normotensive sample did not differ by either family history of hypertension or family history of cardiovascular disease. This was probably due to the low levels of cortisol produced by both groups, as discussed earlier.

Previous research also suggests a strong association between proinflammatory cytokines and many elements of cardiovascular disease (Ross, 1999; Libby et al, 2002; Fahdi et al, 2003; Kop & Gottdiener, 2005). However, to date, studies have not examined the direction of this relationship. The present study applied a family history design to examine whether those at greater risk of developing cardiovascular disease in the future had greater stress-induced IL-6 increases. Results revealed that participants classified as family history positive had similar stress-induced IL-6 responses compared with those classified as family history negative. For exploratory reasons, family history risk was reclassified from negative versus positive risk to low or moderate versus strong risk. Using this less sensitive categorisation, it was discovered that those with a strong family history risk of cardiovascular disease had significantly greater IL-6 responses to the stressors than those with a low or moderate risk. Although the results are not as definitive as those using the more sensitive classification system, they are still important as they demonstrate that stress-related increases in IL-6 may be proactive in the development of future cardiovascular disease.

Recent evidence suggests that interleukin-6 stress responses are predictive of ambulatory blood pressure at 3 year follow-up, and may contribute to future disease development (Brydon & Steptoe, 2005). Several mechanisms may explain the relationship between stress-induced increases in interleukin-6 and hypertension development. First, the sympathetic nervous system and the HPA axis are activated in hypertension, and IL-6 has been shown to stimulate these systems (Papanicolaou et al,

1996). Second, IL-6 disrupts haemostasis, and haemostasis is often disrupted in hypertension (Makris et al, 1997). Third, IL-6 affects a number of mechanisms involved in hypertensive arterial remodelling. These include proliferation of the smooth muscle cells, vascular fibrosis, apoptosis, low-grade inflammation and oxidative stress (Intengan & Schiffrin, 2001). IL-6 stimulates proliferation of vascular smooth muscle cells *in vitro* and increases vessel wall synthesis of collagen (Duncan & Berman, 1991). In addition, IL-6 stimulates the acute phase inflammatory protein, C-reactive protein, which promotes endothelial dysfunction (Pasceri et al, 2000). IL-6 also increases the expression of angiotensinogen, the precursor of angiotensin II. Notably angiotensin II is a potent vasoconstrictor that promotes vascular remodelling, and plasma angiotensinogen concentrations were found to be positively correlated with blood pressure in healthy men (Schorr et al, 1998). Finally, IL-6 may promote disorders of the cardiovascular system by modulating platelet activity and nitric oxide concentrations. Platelet activity and aggregation are increased in hypertension and this contributes to both altered haemostasis and vascular remodelling (Minuz et al, 2004), while IL-6 may also be responsible for increased blood pressure by inhibiting nitric oxide-mediated vasodilatation (Orshal & Khalil, 2004).

A summary of the evidence suggests that, over time, increased stress-induced concentrations of interleukin-6 may result in disorders of the cardiovascular system. This may explain why individuals at greater risk of cardiovascular disease in this study demonstrated larger IL-6 increases to stress. In accordance with these findings one must consider that observed individual variation in IL-6 reactivity may be genetically determined. A common polymorphism in the promoter sequence Gly174Cys of the *IL-6* gene, resulting in increases in plasma concentration of IL-6, has been associated with increased blood pressure in healthy men (Humphries et al, 2001), while hypertensive

response to acute psychosocial stress was blunted in IL-6 knockout mice compared with wild type controls (Lee et al, 2004). Future studies investigating individual stress responsivity and cardiovascular disease development would benefit from gene expression analysis.

#### ***4.4.3.5 Methodological Limitations of Family History Classification***

The null results observed may be attributed to a number of methodological difficulties associated with classification of family history groups. First, problems which were discussed with the classification of hypertension risk may also be applicable to the categorisation of cardiovascular disease risk. For example, the young age of the sample resulted in few parents with evidence of cardiovascular disease-related disorders. In addition, the reliability of reported grandparent incidence of cardiovascular disease-related disorders needs to be considered. Although grandparent age was not ascertained directly, it can be estimated that these people would be in their 70's or 80's. The incidence rates reported in section 4.3.3 are therefore very low for that particular age group. Just 12.4 % of the grandparents were reported to suffer from high cholesterol. This is strikingly different from the 2003 national average of 69.3 % (Joint Health Survey Unit, 2005). Similarly, incidence of heart disease (23.4 %) among grandparents was lower than the 2003 national average of 30.8 % (Office for National Statistics, 2005). Although the low incidence of grandparent cardiovascular disorders reported in this study could theoretically be due to a healthy sample, it needs to be considered that self-reports by parents may be inaccurate. The reliability of these results may therefore have attenuated some of the findings.

Second, there were a sizable number of non-responders to the parental questionnaires. This was especially important when information was gathered from just one parent (and subsequently two grandparents). Statistical power would have been

reduced substantially if only subjects with two parents had been assessed. It was decided, therefore, to classify the non-responding partners of parents that did respond with a negative family history. Participants who had neither parent respond were excluded from this analysis altogether. Although this conservative classification ensured that there was a large enough sample to conduct the analysis, it meant that the negative family offspring group may have contained false positives which could have diluted reactivity differences, thereby possibly accounting for some of the null results observed (Silberberg et al, 1999). This may also explain why an effect was found for IL-6 reactivity when classification of family history was slightly less sensitive.

Third, research has suggested that having more family history groups with graded severity, may yield better prediction of hypertension development (Watt et al, 1991; Muldoon et al, 1993). For example, a comparison of three groups is recommended; that is it may be beneficial to compare normotensives that have both parents and all grandparents with no history of cardiovascular disease with, normotensives who have just one parent (and two grand parents), to normotensives that have either parents or all grandparents with a history of cardiovascular disease.

Last, the reliability of the classification system needs to be considered. Information on parents and grandparents was obtained through parental self-report. It is not possible therefore, to ascertain how accurate these details were. For example, it cannot be relied upon that parent's reports of hypertension, raised blood pressure, experience of heart disease or diabetes were correct or up to date. Parents were not asked when they had last been to the doctor or whether they had ever had their blood pressure or cholesterol measured. It may be possible that parents classified themselves as negative, when in fact they did not know. Other studies have suggested that gathering additional information to support these self-reports would be advantageous

(for example, Vögele & Steptoe, 1993). It is proposed that the age at which these disorders were first diagnosed should be accounted for (Higgins et al, 1996), and that examination of relative's physician notes or clinical assessments of parental health status may be useful when detecting differences between offspring reactivity levels (Sausen et al, 1991; Miller, 1992; Schneider et al, 2003). Although this study did try to adhere to these principles (for example, parents were only defined as condition-positive if diagnosis was before age 60); the use of both parental and grandparent information (while useful its own right) would have made it difficult to gather many of the other details suggested, especially considering the parental response rates to a simple questionnaire.

#### ***4.4.3.6 Section Summary***

Taken together, the results suggest that participants with a positive family history of cardiovascular disease have heightened cardiovascular reactivity to, and impaired recovery from stress (in particular elevated diastolic and systolic blood pressure). These effects were also independent of other cardiovascular risk factors the students may have had. Although certain methodological refinements are still needed, the use of a family history design has proved important when determining whether stress responsivity precedes cardiovascular disorders in individuals most at risk. It is known that a positive family history of cardiovascular disease is strongly associated with future disease incidence, even when environmental risk factors are accounted for (Williams et al, 2001b; Andresdottir et al, 2003). The use of a family history design is therefore, a valuable way of identifying those most at risk.

Although the findings from this study are strictly cross-sectional, the use of a family history design does suggest that physiological stress responsivity could contribute to the future development of cardiovascular disease. In accordance with the

model of allostatic load, it can be theorised that, over time repeated cardiovascular responsivity may result in cumulative detrimental alterations in cardiovascular structure and function which could result in plaque formation, atherosclerotic events and endothelial injury in those individuals most at risk (Ross, 1999). It should also be noted that this design is only valid if the excess cardiovascular risk, associated with stress and allostasis, is due to genetic and family effects. If heightened stress responsivity derives from other sources (i.e. current life stress, and not individual differences in responsivity) then the family history classification will be irrelevant. As a result it can be suggested that the study has demonstrated that genetically or family-derived individual differences in stress responsivity may contribute to the future onset of cardiovascular disease.

This suggestion of increased vulnerability to cardiovascular-related disorders is supported further by longitudinal evidence. Several previous studies have reported that heightened cardiovascular reactivity to psychological tasks is associated with future increase in hypertension (Light et al, 1992; Murphy et al, 1992; Matthews et al, 1993; Markovitz et al, 1998; Newman et al, 1999; Stewart & France, 2001); while delayed cardiovascular recovery is also associated with future increases in blood pressure (Borghi et al, 1986; Tanji et al, 1989; Singh et al, 1999). The most effective way to assess whether the association between cardiovascular responsivity and cardiovascular disease exists over time would be to follow the disease status of these students over a number of years.

The findings of the present investigation also reveal that participants with a strong family history of cardiovascular disease had greater IL-6 elevations to stress than those with a moderate cardiovascular risk, even when controlling for other common cardiovascular disease risk factors. Although some reclassification was necessary, these results are of particular importance as this appears to be the first time that the family



history design was employed to determine the predictive nature of cytokine reactivity and risk of future cardiovascular disease. The principles of allostatic load may again be applied to explain the relationship between interleukins and cardiovascular disease. For example, pro-inflammatory cytokines have, for many years, been associated with heart disease, hypertension, obesity and diabetes (Libby et al, 2002; Fahdi et al, 2003). The use of a family history design makes it possible to suggest that, for vulnerable individuals repeated IL-6 elevation to stress may, over time, place added wear and tear on the body's vascular system, leading to eventual atherosclerosis and cardiovascular dysfunction. The use of the family history design has therefore proved useful in suggesting that cytokine and cardiovascular reactivity may contribute to the future development of cardiovascular disease.

#### **4.4.4 Parental Adiposity and Physiological Stress Responsivity**

Obesity is considered a separate risk factor for cardiovascular disease (McGinnis & Foege, 1993) and other related disorders, including diabetes (Kelley, 1998; Ljung et al, 2000), high cholesterol and hypertension (Eckel, 1997; Heyka, 1998; Melanson et al, 2001). It is believed that physiological stress responsivity may also be related to weight gain. To date, the majority of research has concentrated on the association between obesity and neuroendocrine dysregulation, with numerous studies suggesting a link between adiposity and increased cortisol clearance, higher than average cortisol turn over, and altered cortisol metabolism in adipose tissue (Rebuffe-Scrive et al, 1990; Bujalska et al, 1997; Vicannati & Pasquali, 2000). However, it is unclear at present, whether increased stress responsivity may lead to increased adiposity, which in turn elevates ones risk of cardiovascular disease. Alternatively, it is not clear whether physiological dysfunction is the mediatory factor between obesity and cardiovascular disease. The investigation used a family history design to determine whether

individuals who are at higher risk of weight gain in later life had elevated physiological reactions to stress. The study also investigated cardiovascular and cytokine responsivity as these factors are often neglected in adiposity research. In addition, physiological reactivity was assessed in relation to student's own adiposity in order to detect whether overweight individuals are already be prone to physiological dysfunction (and subsequently increased risk of cardiovascular disease in later life).

#### ***4.4.4.1 Parental Adiposity and Cardiovascular Responsivity***

Participants classified as most at risk from future weight gain had parents with either a large body mass index or waist measurement. The results revealed that cardiovascular reactivity to, or recovery from, the two stress tasks did not differ significantly according to participant's risk of future adiposity. Although, female participants whose fathers had a large waist measurement had greater systolic blood pressure reactivity to the first task than those females whose fathers had a smaller waist measurement. This indicates that increased systolic blood pressure reactivity precedes the development of adiposity in those most at risk of future weight gain. The mechanisms supporting this proposal need to be considered.

It is recognised that insulin resistance and dyslipidaemia may be involved in the relationship between elevated cardiovascular reactivity and increased adiposity (Jern et al, 1992). However, evidence is less clear as to why some individuals may be more reactive to psychological stressors than others. The fact that this study demonstrated that a positive family risk of increased adiposity was related to elevated blood pressure reactivity suggests genetic influences may account for individual variability in vascular responsivity. It has already been mentioned that polymorphisms in codon 16 (Arg16Gly) of the  $\beta_2$ -adrenergic receptor gene (*ADRB2*) are related to variations in transmitter synthesis, transmitter release and reuptake, enzymatic degradation and

receptor activation which can alter vascular activity. It is now believed that persons with this polymorphic genotype often exhibit obesity-related phenotypes as well. For example, amino acid polymorphisms in *ADRB2* (Arg16Gly) have been associated with increased waist to hip ratio (Pereira et al, 2003), male body mass index greater than 35 kg/m<sup>2</sup> (Ukkola et al, 2000) and systolic blood pressure and elevated central distribution of body fat (Rosmond et al, 2000). Genetic analysis is still in its infancy and the role of  $\beta_2$ -adrenergic receptor genes in the relationship between vascular dysfunction and adiposity remains controversial (Hayakawa et al, 2000). Nonetheless, polymorphisms of the  $\beta_2$ -adrenergic receptor gene may explain to some extent why certain individuals are more vulnerable to the elevated systolic blood pressure and elevated adiposity risk. Future research would need to investigate the genotypes of participants with differential blood pressure responses and obesity risk in order to understand the involvement of genetic variation in health-damaging phenotype expression.

Although genetic variation in stress responsivity may account for the association found between systolic blood pressure and increased risk of future adiposity, it is still unclear why other elements of the cardiovascular system were not related to parental adiposity in the same way. This may be because only a few cardiovascular risk factors are associated with DNA sequence variation or more likely because various methodological limitations exist.

First, waist circumference suffers from measurement error (Dalton et al, 2003). Of those parents who returned the questionnaire, 20 % of mothers and 10 % of fathers failed to complete their waist measurement. This may be due either to lack of time, not knowing the measurement or perhaps embarrassment. It is also impossible to tell how accurate or up to date these measurements were; because so few measurements were reported to one decimal place or more it seems unlikely that many waist circumferences

were measured specifically for the questionnaire. Factors such as waist measurement are also open to social desirability bias. Second, the age of the sample may have again been problematic. The fact that parents were relatively young, healthy and possibly from a high social status (as they had children at university) could have lead to the restricted range of adiposity measures being obtained. The descriptive cross-sectional nature of the study also meant that equal numbers of over-weight and average-weight parents could not be pre-selected.

The results of the study also revealed curvilinear relationships for cardiovascular variables and parental adiposity, including diastolic blood pressure, heart rate and heart rate variability. The curvilinear results were mainly observed as high responsivity of the 'low adiposity' group. Although an explanation for these results is not known, it is believed that the small number of male participants or the probability of a number of highly reactive outliers in the low adiposity group may have contributed to these chance effects.

Findings from this study suggest that increased cardiovascular responsivity contributes to future development of cardiovascular disease, and possibly future weight gain. However, it is not clear whether increased reactivity may increase one's chances of becoming obese, which would then heighten one's risk of cardiovascular disease. Or whether being overweight could lead to cardiovascular dysregulation which in turn would increase one's risk of developing cardiovascular disease. The present study was not designed to investigate this alternative hypothesis specifically. As a results it is not possible to determine the exact relationship between stress responsivity, weight gain and risk of cardiovascular disease from the present results. However, findings from previous literature do suggest that cardiovascular reactivity is heightened when one is

overweight (Barnes et al, 1998; Davis et al, 1999; Waldstein et al, 1999; Steptoe & Cropley, 2000; Nagai et al, 2003).

In an attempt to investigate this concept further, the study examined the stress response of students according to their own adiposity. The findings in general failed to show that student's own weight was related to cardiovascular responsivity. Only diastolic blood pressure at baseline and in reaction to task one appeared to be associated with student's own waist to hip ratio. Restricted adiposity range may explain these null results, as students may not yet have sufficient weight gain to affect normal cardiovascular regulation. Future research needs therefore, to follow-up cardiovascular disease incidence of those participants who are currently overweight and have an elevated stress response, in order to compare them with average weight, normally responding participants.

#### ***4.4.4.2 Parental Adiposity and Cortisol Reactivity***

Previous research has concluded that elevated cortisol reactivity is associated with increased body weight, typically central adiposity (Marin et al, 1992; Rosmond et al, 1998; Epel et al, 2000). Due to the correlational design and sample characteristics of these studies, the direction of this relationship between cortisol reactivity and obesity is unclear. A family history design was employed in this study to discover whether elevated cortisol reactivity precedes increased adiposity in those most at risk of future weight gain. It was hypothesised, that students with an increased risk of obesity (defined by heightened parental body mass index or waist circumference) would have significantly greater cortisol reactivity to the two stressors. The findings revealed no association between mother's adiposity and student's cortisol response. Although it was found that female participants, whose fathers had a large waist circumference, produced a stronger cortisol reaction to the tasks than participants whose fathers had

medium or smaller waist measurements; this relationship was also independent of student's own weight, smoking status and baseline cortisol concentrations. These findings extend the results of previous studies and suggest that cortisol responsivity may contribute to future development of obesity.

It is well documented that cortisol contributes to the regulation of adipose tissue differentiation, function and distribution. In excess this can lead to abdominal obesity (Björntorp, 2001). However, the factors which render some individuals vulnerable to elevated cortisol responsivity are less clear. It is believed that most variation in human adiposity is a result of a limited number of common genetic variations that interact with the environment to produce the final phenotype. Two possible pathways have been proposed. First, it is suggested that common familial personality traits may influence cortisol reactivity and risk of future weight gain. However, it seems unlikely in this study that common personality factors present in fathers and daughters should specifically influence female cortisol reactivity and risk of future weight gain. The second mechanism is that genotype may determine individual variation in cortisol clearance, release and reuptake, and receptor activation, efficacy and sensitivity.

Simultaneous analysis of five comparable twin studies suggests a heritability level of 62 % for cortisol release (Bartels et al, 2003). The strongest heritability factor is not basal cortisol levels but dysregulation of diurnal pattern, particularly in response to waking (Kupper et al, 2005). There are also genetic influences of variance in fat distribution, so it is possible that the elevations in cortisol reactivity and increased risk of central adiposity found in this study may be genetically linked. At present, polymorphisms in three genes have been identified.

First, variants of the glucocorticoid receptor (*GR*) gene have been implicated in individual variation in cell sensitivity to glucocorticoids. For example, individuals with

a common *BclI* restriction fragment length polymorphism in the *GR* gene have been shown to exhibit increased salivary cortisol response to stress (Wüst et al, 2004). This polymorphism has also been associated with insulin resistance in obese women (Weaver et al, 1992), abdominal fat in marginally obese men and women (Panerelli et al, 1998), and body mass index, waist to hip ratio, leptin and cortisol responses to a standardised lunch (Rosmond et al, 2000).

Second, individual differences in cortisol stress reactivity may result from genetic variation in the gamma-aminobutyric acid (GABA) receptors. Centrally, cortisol secretion is partially controlled by GABA, which is the main inhibitory neurotransmitter, which acts by binding to the GABA<sub>A</sub> receptors. Evidence by Rosmond et al (2002a) suggests that Thr1519Cys polymorphism in the alpha 6 subunit of the GABA<sub>A</sub> receptor (GABA<sub>Aα6</sub>) and corresponding gene is related to the predisposition of hypercortisolism and abdominal obesity in 284 Swedish men.

Third, serotonin may be involved in the genetic link between individual cortisol reactivity and risk of future obesity. Evidence suggests that cortisol secretion is regulated by central 5-HT<sub>2A/2C</sub> receptors (Rittenhouse et al, 1994). Recently it has been discovered that *MspI* restriction fragment length polymorphism in the promoter region of the *5-HT<sub>2A</sub>* gene (Gly1438Ala) is related to higher body mass index and waist to hip ratio (Rosmond et al, 2002b).

Although the current study did not investigate participant genotypes, research highlighting individual genetic vulnerability with the association of cortisol reactivity and adiposity phenotypes, suggests that the results of this particular study could be genetically determined. In summary, obesity and weight gain have strong heritable properties deriving from the brain and periphery. The use of a family history design has highlighted that those female participants with strong cortisol responses may be at

increased risk of future weight gain (as indicated by father's waist circumference). This, in turn suggests that genetically vulnerable individuals may experience the cumulative effects of cortisol reactivity that, over time result in increased weight gain and an added risk for cardiovascular disease. The investigation of these specific genetic polymorphisms in relation to stress reactivity and obesity development is needed in order that individual-specific treatments, which target the affected neurotransmitter systems, can be developed.

Despite this encouraging finding, several other questions remain unanswered. For instance, because only a small sample of male participants was assessed, it is still unclear how cortisol reactivity and future weight gain affect this group. Also, it is not known why the relationship was present for cortisol reactivity and father's, but not mothers, adiposity. Lastly, the fact that this relationship was observed only for cortisol reactivity and parental adiposity and not student's own weight needs to be considered. Due to these uncertainties, alternative explanations for the observed relationships between student cortisol reactivity and parental adiposity need to be explored.

The first explanation includes methodological limitations similar to those mentioned in Section 4.4.3.5. For example, a small healthy sample, with restricted weight range and self-reported parental adiposity measures may have obscured the results. It may be the case that individual variability in student's own adiposity was not sufficient to detect associations with cortisol reactivity in this particular group. Second, restricted individual variability in cortisol response may have accounted for some of the null results (this is discussed in detail in Section 4.4.1). Third, it must also be considered that cortisol reactivity and adiposity may only become associated when the individual is overweight, this would explain the null results found for student adiposity



and cortisol reactivity in this particular investigation. The mediation of obesity and cardiovascular disease by cortisol reactivity, would explain the apparent impairment of 11 $\beta$ -HSD1 enzymes, disruption of cortisone reactivation and leptin resistance found in obese individuals (Andrew et al, 1998; Stewart et al, 1999; Rask et al, 2001; Leal-Cerro et al, 2001).

#### ***4.4.4.3 Parental Adiposity and Interleukin-6 Reactivity***

Cytokine concentrations are known to increase under stressful conditions and are associated with obesity (Mohamed-Ali et al, 1997; Yudkin et al, 1999; Brydon et al, 2004). However, until now, work on individuals at risk of future adiposity, and cytokine reactivity has been neglected. It is believed that this is the first time a family history design has been used to examine the relationship between cytokine activation and risk of weight gain. It was hypothesised that participants at greater risk of future weight gain would have greater IL-6 increases to stress. Results revealed that IL-6 concentrations did increase significantly in reaction to the stressor; however, these responses did not differ according to parental adiposity. Similarly, the results of the present investigation failed to show any relationship between IL-6 responsivity and student's own adiposity levels; although this may be due to the young, healthy sample tested. Longitudinal evidence is needed, therefore, to clarify the relationship between proinflammatory cytokines, adiposity and cardiovascular disease.

#### ***4.4.4.4 Section Summary***

Previous literature suggests that a strong relationship exists between adiposity and cardiovascular disease and physiological responsivity and adiposity. However, the evidence is not clear as to whether heightened stress responsivity is predictive of future weight gain and increased cardiovascular disease risk as a result of this weight gain, or

whether cardiovascular dysregulation and subsequent cardiovascular disease risk only arises once a person is overweight. With the use of a family history design, the present study endeavoured to investigate whether cardiovascular, neuroendocrine and cytokine responsivity were related to future risk of excess weight in a sample of young, healthy individuals. The results found that female systolic blood pressure and cortisol reactivity could be a contributory factor to future risk of visceral adiposity and cardiovascular disease. However, because the present study was not designed to specifically assess alternative hypotheses, it cannot be discounted that cardiac changes which affect future cardiovascular disease development may only become apparent when one is overweight. Although an attempt was made to assess student's own adiposity and physiological reactivity, results failed to show that overweight individuals had greater physiological stress responses, possibly due to sample characteristics. It is concluded that, although the relationship between physiological responsivity and adiposity is still uncertain, the study managed to emphasise the importance of this factor in obesity research.

#### **4.4.5 Limitations of the Current Investigation**

The present study revealed that individuals at greater risk of cardiovascular disease development had exaggerated cardiovascular and cytokine responses to laboratory stress; and that female cortisol and systolic blood pressure reactivity was related to current and future risk of increased adiposity. The investigation also controlled for student's own baseline physiological levels, body mass index, waist to hip ratio and smoking status. However, despite a determined effort for methodological rigour, several limitations of the current investigation must be considered.

First, although the study design was strengthened by the use of medical information from two generations of relatives, several problems concerning family

history classification persisted. These are outlined in detail in Section 4.4.3.5; in summary, the present study would have benefited from a larger, more diverse student and parental sample. This could be attained by examining more male participants, those from different social status or young participants that were not attending university.

Second, although post-task related interleukin-6 production did differ by family history risk of cardiovascular disease, only an 8 % increase in cytokine concentration was observed for this sample. Despite the fact that this modest increase compares favourably to previous findings (Steptoe et al, 2001) it is difficult to tell whether concentrations would have continued to rise after the experiment had ended. Had a longer post-task period been assessed, larger and more diverse IL-6 concentrations may have been produced, possibly providing more definitive results.

Third, although the study was designed to assess participants up to 90 minutes post-task, it was not possible to examine the recovery phase for cortisol or cytokine production. Findings demonstrate that cortisol and IL-6 had not decreased by the end of the session. Previous studies have also tended to neglect the role of neuroendocrine and cytokine recovery in cardiovascular disease investigation (possibly for the same methodological reasons). It is unfortunate therefore, that this study was unable to add insight into this area of stress physiology research.

Fourth, conclusions from the present study are based on the assumption that a positive family history will eventually lead to development of the disease. Although this may be true in the majority of cases, as several studies confirm (Borghi et al, 1986; Tanji et al, 1989; Light et al, 1992; Murphy et al, 1992; Matthews et al, 1993; Markovitz et al, 1998; Singh et al, 1999; Newman et al, 1999; Stewart & France, 2001), it has to be considered that not all individuals with a positive family history of cardiovascular disease or obesity will go on to develop the condition. The possibility of

false positive results means that interpretation of the study's findings, which suggest that physiological responsivity may contribute to disease development, needs to be considered with a degree of caution. To address this issue, longitudinal examination of this sample investigating the association between physiological reactivity and disease development at follow-up is needed.

Fifth, the study was designed to investigate the relationship between stress responsivity and risk of cardiovascular disease, hypertension and adiposity. The results, however, do not determine whether group differences for each classification are mutually exclusive. Only one third of participants classified with a positive risk of hypertension were classified with a high risk of cardiovascular disease. This means that 48 individuals who were originally classified without risk of hypertension had a positive family history of cardiovascular disease. This suggests that between-group differences found for physiological reactivity may be explained by the risk associated with increased incidence of other cardiovascular disease-related factors present in these people. Further investigation is needed to determine if this is true or alternatively, whether reclassification of these people may have strengthened the group differences for hypertension risk which may have been present had the parental sample been older or clinically assessed.

Sixth, although the findings suggest that cardiovascular and cytokine reactivity precedes cardiovascular disease onset and cortisol reactivity precedes weight gain, the cross sectional nature of the design means that the causal direction of this relationship cannot be ascertained. Future investigation would benefit from the use of experimental (and possibly pharmacological) manipulations to confirm that interruption of these proposed pathways will alter health outcomes.

Lastly, the study did not explore additional third variables which might explain the significant relationship between a positive family history and elevated physiological responsivity. Psychological characteristics are known to independently increase physiological responsivity and are also known to differ by family history (Semenchuk & Larkin, 1993). The present study was careful to examine the role of task appraisals in the relationship between family history risk and physiological responsivity. Subjective task appraisals were found not to differ by family history risk in this study, so were eliminated as possible mediators between positive family history and physiological responsivity. Research has demonstrated that several other behavioural factors, including avoidant coping, anger inhibition, hostility and defensiveness may be involved in the relationship between stress responsivity and development of cardiovascular disease (Ernst et al, 1990; Jorgenson et al, 1992; Vögele & Steptoe, 1993; Shapiro et al, 1995; Schwerdtfeger et al, 2005). The assessment of behavioural responses and sample characterises is important in future analysis of stress responsivity and risk of cardiovascular disease development.

#### **4.4.6 Implications for Future Research**

Cardiovascular disease and its risk factors, including obesity, are becoming increasingly prevalent in western societies (Ryan, 2002). The current study attempted to examine the stress-related factors which increase ones risk of developing these conditions. Identifying the factors which increase disease vulnerability is important to patients and clinicians alike. The use of a family history design identifies individuals most at risk and provides an opportunity for early interventions and improved control of hypertension, high cholesterol, diabetes, obesity and heart disease.

The Reykjavik Cohort Study reported that vulnerable individuals often run a 'double hazard' of developing cardiovascular disease. They are prone to cardiovascular

disease both through their family background and their current lifestyle, which, in addition to heightened physiological responsivity, often includes poor diet, smoking and a lack of exercise (Brenn & Njolstad, 1998). Early identification of individuals with increased genetic predisposition and altered stress physiology means they can then be targeted for risk reduction programmes which are tailored to include increased activity, controlled diet, smoking cessation and stress reduction (Eisenmann, 2003). Research investigating the effectiveness of preventative measures in normotensive populations that have been screened for disturbed physiological responsivity is warranted. This is because a maladaptive response to repeated psychological stress in genetically predisposed individuals might be an early psychophysiological marker in determining which persons will eventually develop cardiovascular disease. Physicians should therefore consider screening these individuals in order to allow for pre-emptive treatment in the early, pre-clinical phase of the disease. Despite the importance of early detection, the EUROASPIRE II Study found that premature coronary artery disease did not encourage doctors to increase risk factor screening in family members (De Sutter et al, 2003). Increasing physician awareness of these factors is therefore crucial as well.

Longitudinal treatment-outcome studies on vulnerable individuals, using anti-stress inoculation, biofeedback therapy, relaxation techniques and / or drug therapy to reduce physiological stress arousal might identify whether the development of cardiovascular disease can be altered in genetically predisposed individuals. In the mean time it is important for physicians to inform patients that not everyone with a family history of cardiovascular disease will develop the condition, but preventative steps to alter lifestyle and reduce stress among the most vulnerable should be encouraged.

#### **4.4.7 Conclusion**

Based on the theory of allostatic load, the previous two studies in this thesis demonstrated an association between physiological dysregulation and impaired health. However, due to the correlational nature of their designs, the contribution of stress responsivity to future disease development could not be established. This third study was designed to explore whether stress responsivity was heightened in individuals with an increased risk of future disease development. With the use of a family history design to identify those most at risk, the investigation discovered that normotensives with a positive family history of cardiovascular disease or increased adiposity (but not hypertension) had significantly elevated cardiovascular, neuroendocrine or cytokine responses to psychological stress. Although certain methodological limitations were encountered, these results suggest that heightened blood pressure, heart rate, heart rate variability and interleukin-6 reactivity precedes the onset of cardiovascular disease in participants most at risk from cardiovascular disease, and that cortisol and systolic blood pressure reactivity may be related to future risk of increased central adiposity. The results of the present investigation are important as they potentially identify genetically vulnerable individuals who would benefit from early risk reduction strategies designed to lower their chance of future cardiovascular disease development.

## **Chapter 5: Acute Inflammation and Negative Mood: Mediation by Cytokine Activation (Study 4)**

### **5.1 Introduction**

#### **5.1.1 Rationale**

The three studies presented so far have explored the cumulative effect of stress perception and physiological responsivity on health. All three studies are based on the theory of allostatic load which postulates a causal relationship between stress and pathological outcome. However, examination of the association between physiological activation and ill-health across the life course would be incomplete without consideration of the bi-directional nature of physiological responsivity systems. The linear relationship between the central nervous system and regulatory systems implies that stress appraisals centred in high-order brain areas, such as the limbic system affect physiological mechanisms through the HPA axis, immunological and autonomic pathways, which, if persistent over time may be detrimental to health. In contrast, a bi-directional relationship suggests that the same regulatory pathways may also influence these high-order brain regions directly, thus affecting psychological wellbeing and health outcome (Besedovsky & Del Rey, 1996). This final study attempts, therefore, to extend the theory of allostatic load by investigating the existence of a bi-directional relationship between complex human central nervous system function, physiological regulation and health.



### **5.1.2 Cytokine Induced Sickness Behaviour**

There is growing recognition that the immune system does not act in isolation and that communication pathways exist between the inflammatory response and the brain, often resulting in sickness behaviour. In this way, infectious and inflammatory diseases are associated with numerous behavioural disturbances including malaise, fever, lethargy, anorexia, hypersomnia, inactivity, impaired cognition and anhedonia or depressed mood (Larson & Dunn, 2001). Once commonly dismissed by physicians and merely regarded as an unpleasant side-effect of illness, it is now believed that these psychological and behavioural changes form part of a highly organised strategy of natural homeostatic reaction used to fight infection (Hart, 1988; Vollmer-Conna, 2001). For example, higher body temperature which is achieved during fever stimulates proliferation of immune cells and is unfavourable for the growth of many bacterial and viral pathogens. In addition, the reduction of zinc and iron levels in plasma that occur with fever decrease the availability of these vital elements for growth and multiplication of micro organisms. Similarly, lethargy and depressed mood are advantageous to the infected individual as they act to induce a state where energy can be conserved for recovery (Dantzer, 2004). In recent years, attention has turned to the identification of possible physiological pathways which might mediate this relationship. It is now believed that this process is triggered by proinflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF $\alpha$ ) (Konsman et al, 2002).

### **5.1.3 Cytokine Induced Sickness Behaviour and Depressed Mood**

One of the main factors related to cytokine induced sickness behaviour is the presence of depressed mood. Although evidence relating cytokines to sub-clinical

depressive symptoms is inconsistent (Haack et al, 1999; Kop et al, 2002; Miller et al, 2003; Steptoe et al, 2003b), elevated cytokine levels are associated with major depression (Irwin, 2002). For example, cancer patients with depression have higher plasma concentrations of IL-6 than healthy controls or cancer patients without depression (Musselman et al, 2001), and depressive symptoms are common in conditions associated with cytokine activation, including autoimmune disorders, stroke, trauma, cardiovascular diseases and Alzheimer's disease (Yirmiya, 1997; Yirmiya et al, 1999; Pollmächer et al, 2002). This link between cytokine secretion and depression is particularly important considering the high prevalence of depressed mood in old age. However, the causal direction of these associations is, at present unclear.

It has been argued, in a sense similar to the theory of allostatic load, that depressed mood stimulates inflammatory responses, and that over time this mechanism might contribute to conditions such as acute coronary heart disease (Black & Garbutt, 2002). There is also evidence that anti-depressant treatments which target the neurochemical basis of depression have anti-inflammatory effects (Musselman et al, 2001; Castanon et al, 2002). However, it is now conversely believed that illness or stress-induced inflammatory response may actively contribute to depressive mood and negative affect. The study of this bidirectional relationship is crucial therefore when trying to gain a more comprehensive understanding of the outcomes of physiological stress responsivity. Studies of patients with cancer indicate that immune therapy using IL-2 or interferon- $\alpha$  can lead to depressed mood that correlates with the magnitude of endogenous cytokine responses (Capuron et al, 2001; Beratis et al, 2005), and depressive symptoms have also been reported following interferon- $\alpha$  treatment of hepatitis C (Bonaccorse et al, 2001).

However, these observations do not provide a useful model of the natural phenomenon of sickness behaviour as therapeutic use of cytokines typically employs very high concentrations of the proteins (Vollmer-Conna, 2001; Pollmächer et al, 2002). Results from clinical samples also may not generalise to the population at large since they involve participants with already compromised immune systems. To date, few studies have documented mood disturbances associated with infection-induced illness in humans. Those that have (e.g. Westley-Wise et al, 1996; Vollmer-Conna et al, 1997; Capuron et al, 1999; Smith et al, 2000; Vollmer-Conna et al, 2004), report illness-associated changes in fatigue, mood and cognitive ability; however the direct effects of cytokines are often not assessed and infections are rarely documented serologically. A more controlled and predictable inducer of inflammatory response is therefore required to understand the relationship between inflammation and depression. (Capuron et al, 1999).

#### **5.1.4 Models of Cytokine Induced Depressed Mood**

##### **5.1.4.1 Endotoxin Models**

In order to gain more control over the inflammatory response, research has begun to manipulate acute transient inflammatory responses through vaccination and injection of active endotoxins in humans. The acute effect of an infectious stimulus upon mood in healthy volunteers was investigated by Reichenberg et al (2001). Using *Salmonella abortus equi* endotoxin as a model of experimental inflammation, this double-blind placebo-controlled study observed a 50 to 100 fold increase in IL-6 and TNF $\alpha$  concentrations within 1-4 hours of endotoxin, but not placebo administration. Significant increases in anxiety and depressed mood were reported in the endotoxin but not placebo group. Although endotoxin had no effect on blood pressure, heart rate or

reported sickness, it did cause an increase in body temperature and a marked reduction in food intake. This made it difficult to determine whether behavioural and mood changes were due to the presence of inflammatory challenge, or were caused by the mild illness induced by the endotoxin itself. The key issue now, is to explore whether cytokines communicate with the brain in the absence of overt physical symptoms of illness.

#### **5.1.4.2 Vaccine Models**

In light of these findings, a milder model of experimental inflammation, namely typhoid (*salmonella typhi*) vaccination was introduced by Professor Steptoe's group (Strike et al, 2004). Typhoid vaccination has previously been shown to produce a 4-6 fold increase in IL-6 and a 30 fold increase in IL-1Ra within 2-3 hours of administration (Hingorani et al, 2000; Kharbanda et al, 2002). Results indicated that typhoid vaccination led to a significant decline in mood over an 8 hour period in comparison with placebo, without inducing any physical symptoms or temperature increase. It is believed, therefore, that the use of vaccination rather than endotoxin may afford better insight into changes of mood state in response to immune challenge in healthy volunteers. However, the recent study by Strike et al (2004) did not include measures of cytokines, so it was not possible to directly assess the influence of inflammatory responses on mood changes. Despite this, the results of Reichenberg et al (2001) and Strike et al (2004) appear to suggest that strain on the body brought about by sickness induced cytokine activation may go on to alter mood through pathways linked to complex central nervous system function in the limbic system. This effect would suggest a bi-directional relationship between bodily stress, physiological mechanisms and health outcome, thus adding a new dimension to the theory of allostatic load.

The present study was designed, firstly to replicate previous findings on the effects of typhoid vaccination on subsequent mood, and secondly to assess the mediating role of cytokine responses. The study adopted an experimental double-blind placebo-controlled intervention method in order to ascertain the causal direction of this relationship without concern about reverse causation or third-factor explanations. It was hypothesised that typhoid vaccination (in comparison with placebo) would induce significant increases in IL-6, IL-1Ra and TNF $\alpha$ , teamed with a decline in mood, in the absence of any change in systemic body temperature or symptom ratings of illness.

### **5.1.5 Stress and Cytokine Induced Depressed Mood**

Strain on the body's resources does not occur solely through pathological or pharmacological challenge (such as infection or vaccination). As the previous three studies have outlined, the body's resources may be depleted by stress. There is growing evidence that proinflammatory cytokines are responsive to psychological stress, and in humans, several forms of acute, chronic or episodic life stress are accompanied by elevated circulating levels of inflammatory cytokines. For example, IL-6 levels increase more rapidly over time in caregivers than controls (Kiecolt-Glaser et al, 2003), are elevated in individuals suffering from post-traumatic stress disorder (Maes et al, 1999) and in university students experiencing examination stress (Maes et al, 1998). Research has also shown an association between examination stress, elevated IL-6 production and negative mood (Kang & Fox, 2001). Similarly, acute psychological stress has been shown to stimulate increased concentrations of plasma TNF $\alpha$ , IL-6, and IL-1Ra in healthy individuals (Goebel et al, 2000; Steptoe et al, 2001; Brydon et al, 2004).

Previous research has found that negative changes in mood following typhoid vaccination were correlated with chronic stress, financial strain and job demands. This

suggests that long term stress might influence cytokine response to vaccination by moderating mood state (Strike et al, 2004). However, the impact of stress on acute cytokine and mood response to vaccination has yet to be investigated. The secondary aim of this investigation was therefore to explore the relationship between chronic stress, mood response, cytokine production and typhoid vaccination.

### **5.1.6 Cytokine and Glucocorticoid Interactions and Depressed Mood**

When investigating the effect of cytokine-induced depressed mood it is also important to consider that the immune system does not act alone and is, in fact, mutually interactive with neuroendocrine function (Black, 1994a; Black, 1994b; Straub et al, 2000). The onset of depression-related sickness behaviour may therefore be mediated by sympathetic nervous system activation (Torpy et al, 2000; Capuron & Dantzer, 2003). For example, proinflammatory cytokines may influence central neurotransmitters such as serotonin (Myint & Kim, 2003) and also directly stimulate the HPA axis; thus resulting in the release of adrenocorticotrophic hormone, corticotrophin releasing hormone and cortisol (Besedovsky & Del Rey, 1996; Turnball & Rivier, 1999). Glucocorticoids in turn negatively control cytokine production and by this mechanism are able to shut down inflammatory processes to prevent host destruction due to prolonged immune activity (Sapolsky, 2000). Depression is often associated with a dysregulation of the HPA axis characterised by increases in basal levels of adrenal glucocorticoids and a reduced potency of dexamethasone to suppress adrenal glucocorticoids (Gold et al, 1996). A positive relationship between IL-1 $\beta$ , IL-6 and cortisol has also been found in depressed patients (Maes et al, 1993a; Maes et al, 1993b), while reports show that proinflammatory cytokines are potent activators of HPA activity, and alter monoamine activity within the hypothalamic paraventricular nucleus and limbic system (Anisman & Merali, 1999; Dunn, 2001). However, the exact

nature of this interaction, and subsequently its effect on mood is not known. It may be the case that under different conditions glucocorticoids could enhance inflammatory and immune functions at one concentration and inhibit these functions at another (Black & Garbutt, 2002). In order to explore this relationship further the present study aimed to investigate the effect of cortisol on post-vaccination mood.

## **5.2 Method**

The results of this study have been published in *Brain Behavior and Immunity* (please refer to reprints at the end of the thesis).

### **5.2.1 Participants**

Thirty male student volunteers aged between 18 and 30 years (mean  $23.3 \pm 3.5$ ) were recruited from University College London (see Appendix XXIII for participant information sheet). Participants were all non-smokers, were not on regular medication, had not taken aspirin, ibuprofen or antibiotics in the last 10 days and had no recent mental or physical illness or a typhoid vaccination within the last 6 months. On the morning of the study all participants felt well, reported no recent stressful events and had refrained from caffeine, alcohol and physical exercise for 12 hours before the start of the study. Informed consent was gained from each participant and ethical approval was granted by UCH Medical Research Ethics Committee (Appendix III).

### **5.2.2 Measures**

#### **5.2.2.1 Measurement of Mood**

Mood and symptoms of illness were assessed with a modified, 34-item version of the Profile of Mood States (POMS) (McNair et al, 1981). Six high-loading items

were taken from each of the five original POMS scales (vigour, tension-anxiety, depression-dejection, confusion and fatigue). Four extra items were added to assess symptoms that might be associated with mild infection (fever, aching joints, nausea and headache). Participants were asked to rate their feelings at that moment on a five-point scale ranging from 0 = *not at all* to 4 = *extremely* (Appendix XXIV). Total mood score was calculated (as recommended in the POMS manual) by summing all negative items (tension, depression, confusion, fatigue), and subtracting them from the positive vigour score. Overall mood scores ranged from -80 to 20 (with higher scores indicating a more positive mood). Separate scores were calculated for positive and negative mood scales so that negative mood scores ranged from 0 to 96 (with higher scores indicating a more negative mood) and positive mood scores ranged from 0 to 24 (with higher scores indicating a more positive mood).

#### **5.2.2.2 Anthropometric Measures**

Body temperature was measured with a sub-lingual digital thermometer, and blood pressure and heart rate were measured using an electronic sphygmomanometer (A & D UA779, Tokyo, Japan).

#### **5.2.2.3 Measurement of Chronic Stress**

Chronic stress was assessed with four measures. First, the 14-item Perceived Stress Scale (PSS) (Cohen & Karamak, 1983) documented the degree to which situations within a one-month time frame were appraised as stressful. Item responses ranged from 0 to 4 giving a total in the range of 0 to 56 (with 56 indicating a very high level of perceived stress). Second, financial strain was assessed with the questionnaire originally developed by Pearlin et al (1981) and used in the Whitehall II and other studies (Ross & Huber, 1985; Steptoe & Marmot, 2003). Eight items assessing, for



example, difficulty paying ones bills and being able to replace items such as furniture were presented. Options ranged from 1 (no difficulty) to 3 (very great difficulty). Responses were summed. Total scores ranged from 8 (very low financial strain) to 24 (very high financial strain). The third measure was an index of work demands derived from the demand/control model of work stress used in the Whitehall II study (Marmot et al, 1991). Of the original 25 questions, five relevant questions were selected. The first three assessed general work demands, question four measured consistency and clarity in work demands while the final question assessed the psychological demands of one's university studies. Each of the five questions was rated on a four-point Likert scale ranging from 1 = *almost never* to 4 = *often*. Summed scores ranged from 5 to 20 with a higher score indicating more work stress. Last, a 10-item version of the Undergraduate Stress Questionnaire (USQ) was used (Crandall et al, 1992). The USQ originally comprised 83 'non-school' and 'school' related items particularly relevant and stressful to university students. This 10-item version consisted of five 'non-school' items (e.g. conflicts with housemates, transport problems) and five 'school' items (e.g. exam stress and work-load pressures). The 10 selected items were rated by the original sample as most severe and frequent. Responses were scored in two ways. Frequency of a stressor's occurrence in the last two weeks was scored either 1 = *Yes* or 0 = *No* while severity of a present stressor could be rated as either 1 = *not at all stressful* to 3 = *very stressful*. Overall scores ranged from 0 to 10 for event frequency and 1 to 30 for event severity, with higher scores indicating greater frequency or severity of a stressor (see Appendix XXV for questionnaire booklet).

### 5.2.3 Cytokine Assays

Blood was drawn from the median cubital vein using a 21-gauge butterfly needle into vacutainer tubes containing EDTA as anticoagulant. Whole blood samples (10 ml)

were centrifuged immediately at 1250 xg for 10 min at room temperature. Plasma was removed and frozen at -80 °C until analysis. IL-6 and TNF $\alpha$  was measured using high sensitivity two-site ELISAs from R and D Systems (Oxford, UK). The limit of detection of the IL-6 assay was 0.09 pg/ml, with intra- and inter-assay coefficients of variation of 5.3 % and 9.2 %. For human TNF $\alpha$ , the limit of detection was 0.10 pg/ml and intra- and inter-assay coefficients of variation were 6.9 and 8.4 %. Plasma IL-1Ra concentrations were determined by a commercial ELISA from R and D Systems (Oxford, UK). The assay has a limit of detection of 15 pg/ml and inter- and intra-assay coefficients of variation of less than 10 %. Cytokine assays were performed at University College London by Dr Lena Brydon.

#### **5.2.4 Cortisol Assays**

Saliva samples were collected using Salivette devices (Sarstedt, Inc., Leicester, UK) and stored at -30 °C until analysis. After defrosting, samples were centrifuged at 3000 rpm for five minutes and 100  $\mu$ l of supernatant was used for duplicate analysis involving a time-resolved immunoassay with fluorescence detection (Dressendörfer et al, 1992). Cortisol assays were performed at University College London by Kesson Magid.

#### **5.2.5 Procedure**

The study was performed in a double-blind, randomised, placebo-controlled manner. Participants were assessed individually in a temperature controlled room. To ensure uniformity, the study began at 09:00 h, when baseline measures of body temperature, blood pressure, heart rate, salivary cortisol and mood were taken. Assessment of background stress was also made at this time using the four questionnaires. After a baseline blood sample was drawn, participants were randomly

assigned to one of two experimental groups at a ratio of 2:1 (20 vaccine, 10 placebo). Randomisation was carried out by an investigator who did not assist with participant testing at any stage. Injections of 0.025 mg of *salmonella typhi* capsular lipopolysaccharide vaccine (Typhim Vi, AVEBTI Pasteur MSD), or 0.5 ml of normal saline placebo in identical 2 ml syringes were administered intramuscularly into the non-dominant deltoid muscle at approximately 09:10 h. There were no complications with any of the vaccination or placebo injections. Participants were reassessed at 1.5, 3 and 6 hours post-vaccination. Between sessions participants did not have contact with other volunteers but were free to return to their normal college schedule. At each post-vaccination session, blood pressure, heart rate, temperature, salivary cortisol and current mood were measured. A post-vaccination blood sample was drawn at 3 hours.

### 5.2.6 Statistical Analyses

Questionnaire data were satisfactory for all 30 participants. Group comparisons of baseline mood, symptoms, chronic stress, physiological measures, cytokine IL-6, TNF $\alpha$  and IL-1Ra concentrations were analysed with independent samples t-tests. Changes in mood, blood pressure, heart rate, temperature, cortisol and cytokine concentrations over the study period were analysed using repeated measures analysis of variance (ANOVA) with group (vaccine, placebo) as the between-subject factor and time as the within-subject factor. Follow-up comparisons between individual values were made using Tukey's LSD test. Changes in mood, cortisol and cytokine levels were computed by subtracting the baseline value from later experimental time points. A higher positive change score indicated a greater improvement in mood or increase in cytokine or cortisol concentration. A lower negative change score indicated a greater deterioration in mood or decrease in cytokine or cortisol levels. IL-6 values were skewed so were logged prior to being correlated with changes in mood. The association

between changes in IL-6 and changes in mood was illustrated by dividing the sample into quintiles of mood change. Participants in the lowest quintile reported the most negative mood changes between baseline and 3 hours, while those in the highest quintile had the most positive mood change. The mean change in IL-6 concentration associated with each mood change quintile was plotted. Finally, correlations between chronic stress measures and the changes in mood and cytokine production were computed. Results are presented in terms of means  $\pm$  standard deviations.

## 5.3 Results

Table and Figures to accompany this section can be found starting on page 307. Sample characteristics and baseline measures are summarised in Table 5.1. The vaccine and placebo groups did not differ significantly by age, physiological measures, financial strain, perceived stress, work demands or number and severity of life events. Mood scores indicate that participants demonstrated a slight overall negative mood at baseline. Mood scores did not differ significantly between-groups. None of the participants reported any appreciable symptoms pre-injection.

### 5.3.1 Physiological and Symptom Response to Vaccination

There was a significant quadratic effect over the study period in body temperature ( $F_{1,28} = 7.15$ ,  $p = .012$ ), with lower levels at 1.5 and 3 hours (mean 36.1 °c) than at baseline or 6 hours (36.3 °c). Temperature did not differ by experimental condition. Symptom scores were very low throughout, with only two individuals scoring 2 (moderate) or greater on a single symptom at 1.5 and 3 hours post-injection, and three scoring 2 or greater on a single symptom at 6 hours. The absence of any rise

in body temperature or symptom rating following vaccination indicates that the procedure did not induce illness (Figure 5.1a and 5.1b).

Heart rate showed a significant quadratic effect over the trial ( $F_{1,28} = 11.4$ ,  $p = .002$ ), with lower levels at 1.5 and 3 hours post-injection than at baseline or 6 hours. Vaccine and placebo groups did not differ in this response (Figure 5.1c). There was also a significant time by group interaction ( $F_{1,28} = 5.09$ ,  $p = .032$ ) for systolic blood pressure. This was due to a slightly elevated systolic pressure in the placebo group ( $139.8 \pm 18.1$  mmHg) compared with the vaccine group ( $132.0 \pm 12.1$  mmHg) at baseline. Systolic blood pressure was not increased in the vaccine group at any point in the study. There were no differences between-groups in diastolic blood pressure over the day (Figure 5.1d).

### 5.3.2 Mood Response to Vaccination

The analysis of total mood score over time showed a significant group by time interaction ( $F_{1,28} = 4.76$ ,  $p = .038$ ). This result is illustrated in Figure 5.2. Participants' moods were slightly negative on balance at baseline, but rose 1.5 hours after injection in both groups. The placebo group experienced a sustained elevation in mood for the remainder of the day, whereas the vaccine group showed a marked decline in mood between 1.5 h and 3 h post-injection ( $p = .028$ ). Mood levels continued to fall in the vaccine group until 6 h post-injection ( $p = .009$ ). Follow-up comparisons showed that mood score changes from baseline were greater in the placebo than the vaccine group at 3 and 6 h post-injection ( $t = -2.24$ ,  $p = .034$ ;  $t = -1.92$ ,  $p = .068$ ) (Table 5.2).

Positive and negative mood scales were also analysed. Analyses of negative items revealed that negative mood scores were higher 3 hours post-injection in the vaccine ( $12.8 \pm 7.7$ ) than in the placebo ( $7.40 \pm 5.8$ ) group ( $t = 2.16$ ,  $p = .041$ ). The change between baseline and 3 hours was also smaller in the vaccine than placebo group

( $t = 2.18$ ,  $p = .039$ ). Analyses of the positive vigour scale showed a significant time by group interaction ( $F_{1,28} = 4.44$ ,  $p = .044$ ). Follow-up comparisons showed that while the placebo group's level of vigour did not change significantly during the study, vigour in the vaccine group decreased significantly between 1.5 and 3 hours post-injection ( $p = .048$ ), and was below baseline levels by the end of the study ( $p = .003$ ).

### 5.3.3 Cytokine Response to Vaccination

There was a significant group by time interaction in the analysis of plasma IL-6 ( $F_{1,28} = 9.57$ ,  $p = .004$ ). As shown in Table 5.3, IL-6 concentration doubled between baseline and 3 h post-injection in the vaccine group, and fell slightly in the placebo group. There were no significant changes in  $\text{TNF}\alpha$  or IL-1Ra either within or between the two conditions.

### 5.3.4 Cytokine and Mood Response to Vaccination

Vaccine-induced changes in mood were significantly and negatively correlated with IL-6 concentration changes 3 h post-injection (Figure 5.3). Participants with larger increases in IL-6 showed more deterioration in mood change on the total POMS, 3 h post-injection ( $r = -.42$ ,  $p = .022$ ). This effect was due to associations with the negative mood scale rather than the vigour scale. There was a positive association between IL-6 change and the changes on the negative mood scale of the POMS at 3 h post-injection ( $r = .39$ ,  $p = .032$ ), but no association with changes in vigour. No significant correlations were discovered for  $\text{TNF}\alpha$  or IL-1Ra.

### 5.3.5 Stress and Mood Response to Vaccination

Participants presented relatively low levels of chronic background stress on the five measures employed (financial strain, work stress, perceived stress, number and

severity of life events) (Table 5.1). No significant effect of chronic stress on global mood change was found for either of the experimental groups. Analyses were completed for positive and negative mood items as separate scales. No significant associations were discovered between negative mood change and chronic stress. Product moment correlations between changes in positive mood items and the five stress variables were computed separately for the vaccination and placebo group. No significant correlations were found in the placebo group. In the experimental group, a negative association was discovered between vigour change scores over the 6 h following vaccination and three of the stress measures. These relationships reached significance after the removal of one outlier. A significant negative correlation was found between 6 h vigour change scores and work stress ( $r = -.51$ ,  $p = .026$ ), perceived stress ( $r = -.47$ ,  $p = .04$ ) and severity of life event stress ( $r = -.50$ ,  $p = .03$ ). Financial strain and number of life events were not significantly related to vigour scores. However, it should be pointed out that multiple comparisons were carried out, and if the Bonferroni correction is applied, none of these associations would have remained significant. Separate group correlations were conducted between IL-6, TNF $\alpha$ , and IL-1Ra change levels and the five chronic stress variables. No relationship was present between the change in cytokine production and background chronic stress levels.

### **5.3.6 Cortisol and Mood Response to Vaccination**

Cortisol data were available for 21 participants. No significant main effect or time by group interaction was found for cortisol levels post-injection. A main effect of time was observed ( $F_{1.4, 28.1} = 17.22$ ,  $p = .001$ ). Cortisol concentrations at baseline averaged 5.48 nmol/l in the vaccine group ( $\pm 3.2$ ) and 7.05 nmol/l ( $\pm 6.0$ ) in the placebo group (non-significant). 1.5 h post-injection cortisol levels decreased in both experimental groups as a result of expected time of day effects. At 6 h post-injection,

placebo group cortisol concentrations were no different from 1.5 h post-vaccination but experimental group cortisol concentrations decreased significantly during this same period ( $p = .001$ ) (Table 5.4).

Product moment correlations revealed that total mood change scores at 6 h post-injection were associated with cortisol changes 1.5 h ( $r = -.56$ ,  $p = .026$ ), 3 h ( $r = -.49$ ,  $p = .052$ ) and 6 h ( $r = -.60$ ,  $p = .016$ ) post-injection in the vaccination but not placebo group. These findings indicate that participants with greater decreases in cortisol also presented with larger impairments in positive mood during the testing procedure. Partial correlations between mood change scores at 6 h and cortisol change scores still neared significance when cytokine change scores were controlled for (1.5 h,  $r = .53$ ,  $p = .04$ ; 3 h,  $r = .48$ ,  $p = .07$ ; 6 h,  $r = .59$ ,  $p = .027$ ). Similarly, partial correlations between mood change scores at 6 h and IL-6 change scores also neared significance when cortisol change scores were accounted for (1.5 h,  $r = -.41$ ,  $p = .076$ ; 3 h  $r = -.40$ ,  $p = .092$ ; 6 h,  $r = -.41$ ,  $p = .076$ ). From this it can be suggested tentatively that IL-6 and cortisol could be independently associated with vaccine-induced increases in negative mood. However, no significant relationship was found between cytokine change scores and cortisol production post-injection.

## 5.4 Discussion

Inflammatory diseases are typically associated with depressed mood. This study hypothesised that a mild experimentally-induced inflammatory response would produce a transient decline in mood, and that this association would be mediated by cytokine activation. Due to the possible confounding effects of experimenter and participant expectations, the hypothesis was tested using a double-blind design. The placebo-



controlled design was used to account for spontaneous circadian mood changes which can occur irrespective of vaccination stimuli. An intervention design also allowed causal inferences to be made, without concern about reverse causation or third-factor explanations.

### 5.4.1 Mood Response to Vaccination

The results confirmed the predictions regarding the mood-related effects of inflammatory challenge. Both the placebo and vaccination groups reported an improvement in mood during the first 1.5 h following baseline. This was possibly due to time of day effects or relief at having completed the injection (although this was not assessed directly). By 3 h post-injection, mood in the placebo group continued to rise, before deteriorating slightly later in the day. In contrast, the vaccine group showed a sharp decline in mood state by 3 h post-injection, and mood levels continued to fall below baseline by the end of the study. In addition, participants injected with the *salmonella typhi* vaccination experienced a doubling of IL-6 concentration. As predicted, this elevated production of IL-6 was associated with a greater decline in mood 3 h after baseline. This suggests that the mild inflammatory response is caused by vaccination activated cytokine production, and that the increased production of cytokines subsequently had an adverse effect on mood.

Although the mood differences observed in the present study were significant, they were small. This may have been a consequence of the mild stimulus used. A similar small effect was recorded in a previous study using typhoid vaccination (Strike et al, 2004). This contrasts with Reichenberg et al's (2001) results, where both depression and anxiety were markedly increased in response to endotoxin. The effect observed by Reichenberg et al (2001) may have been due, in part, to the invasive nature of the procedure, which involved venous cannulation and continuous temperature

measurement using a rectal probe. Notably, in that study, anxiety increased in the placebo group as well as in the endotoxin condition, though to a lesser extent.

Reichenberg et al (2001) also found an endotoxin-induced increase in temperature and a reduction in food intake; although the injection did not produce any significant change in subjective ratings of physical sickness symptoms, which is comparable to the findings of this study. In the present study, temperature also decreased during the middle of the day, contrary to expected circadian changes. However, there was no between-group difference in change in body temperature or febrile response, and reported symptoms were consistently low. From this it is contended that the mood changes observed in this study were due to inflammatory responses, and did not result from a toxic effect.

#### **5.4.2 Cytokine Response, Mood and Vaccination**

The IL-6 concentration increase in this study was small, only averaging 106 %. In comparison, Reichenberg et al (2001) found a 10 fold increase in IL-6 production. It has been documented that even low levels of peripheral cytokines can have a profound impact on the human brain (Pollmächer et al, 2002). However, it is thought that the relatively small rise in IL-6 in this study may account for the null results observed when comparing cytokine concentrations to cortisol and stress responses. No changes in IL-1Ra and TNF $\alpha$  were measured and no relationship was found between TNF $\alpha$  or IL-1Ra and mood. This was somewhat unexpected as previous studies using the *salmonella typhi* vaccination have observed large increases in IL-1Ra three hours post-injection (Hingorani et al, 2000; Kharbanda et al, 2002). The lack of effect of *salmonella typhi* vaccination on TNF $\alpha$  is consistent with the findings of Hingorani et al (2000) and Kharbanda et al (2002). Reichenberg et al (2001) did demonstrate an increase in TNF $\alpha$ .

with endotoxin, so it would appear that the attenuated virus used in the vaccine stimulated a much milder inflammatory response. It should be pointed out that the IL-6 response to vaccination was comparable with that elicited by psychological stress. Increases of 97 % and 113 % have been recorded in the two hours following performance of moderately stressful tasks by Professor Steptoe's group (Steptoe & Feldman, 2001; Brydon et al, 2004), while rises in IL-6 levels of about 50 % have been measured in anxious students around the time of a difficult examination (Maes et al, 1998). The IL-6 response to vaccination may therefore be a useful model of cytokine-induced mood responses in psychosocial studies.

### **5.4.3 Cortisol Response, Mood and Vaccination**

The study also aimed to discover whether the relationship between cytokine-induced mood depression was also influenced by documented HPA axis interactions (Besedovsky & Del Rey, 1996). Evidence suggests that cortisol may act as a mediator between IL-6 and mood. However, results from this investigation revealed the possibility of an independent relationship between cytokines, cortisol and mood. These findings, consistent with results from Reichenberg et al (2001) suggest that both cytokine secretion and HPA activation may contribute to vaccine-induced increases in negative mood. This supports the previously suggested role for both cytokines and cortisol in depressive symptoms (Yirmiya, 1997). However, these findings should be interpreted with caution, because contrary to expectation, no relationship between cortisol and cytokine concentration was found; although this may again be due to the low levels of IL-6 detected or the small sample size studied.

#### 5.4.4 Background Stress, Mood and Vaccination

The study also investigated whether background stress would influence mood and cytokine responses to vaccination. Previously, it was shown that the magnitude of mood change following vaccination was associated with financial strain (Strike et al, 2004). There is also evidence from other studies that background stress levels and negative mood states are associated with antibody titres and inflammatory responses following vaccination for influenza, hepatitis A and meningitis C (Vedhara et al, 1999; Burns & Zaudig, 2002; Glaser et al, 2003; Hayney et al, 2003). However, in the current study no association between background stress measures and cytokine production was found. Participants vaccinated with *salmonella typhi* who reported higher work stress, greater perceived stress and more severe life event stress experienced a greater decline in vigour over the 6 h following injection. No such association was observed in the placebo group, which means that a non-specific response caused by negative affectivity reporting bias can be ruled out. However, these correlations were obtained with multiple statistical comparisons, so must be regarded as very weak. It is possible that in this small sample, the variance in the background stress measures was not sufficient to detect robust associations. The influence of physiological responsivity to an acute stressor should also be examined in future investigation. Studies 1 and 3 have demonstrated that individual variability in the stress response is associated with differential health status. Future work needs to explore the possible moderating effects of acute stress on mood response to vaccination.

### **5.4.5 The Bidirectional Relationship between Peripheral Cytokines and the CNS**

Intervention studies, like the present investigation emphasise the bi-directional nature of the relationship between psychological processes and the immune system. Recent research has been dominated by work on the impact of psychological factors on immune function (Segestrom & Miller, 2004); however current opinion suggests that inflammatory responses may actively contribute to depressive mood. Several mechanisms which facilitate the transmission of peripheral cytokine signals to central nervous system targets have been identified. These include direct neural pathways via primary autonomic (vagal) afferents, and humoral mechanisms involving cytokine entry where the blood-brain-barrier is weak or absent. This then triggers the production of secondary messenger targets inducing local cytokine production (Maier & Watkins, 1999). Pathways via the circulatory system include entry at circumventricular sites, where fenestrated capillaries allow plasma passage to occur through gaps in the blood-brain-barrier (Stitt, 1990; Katsuura et al, 1990). Cytokines may also gain access through the blood-brain-barrier via carrier mediated transport (Banks & Kastin, 1991) or they may bind to and affect the metabolism of cerebral vascular endothelium thereby inducing the generation of central mediators. These metabolic effects can alter the permeability of the blood-brain-barrier to other substances including illness mediators that would otherwise be excluded (Cao et al, 1996; Cao et al, 1997).

Data from this investigation suggest that IL-6 could mediate the relationship between the inflammatory process and depression. However, this cytokine is strongly linked to other mechanisms in the inflammatory process. For example, IL-6 acts non-specifically in the body targeting multiple sites. It is therefore easily detected in the blood. IL-6 also has an inhibitory effect on IL-1, and is stimulated by IL-1 $\beta$  (Corwin,

2000a). Subsequently, it is unknown whether the observed effects between mood and vaccination were solely due to mediation by IL-6, or whether other more specific and less prolific substances are involved. Studies using IL-6 knock-out animals have demonstrated that this cytokine plays an important role in the association between mood change and inflammation (Dantzer et al, 1999); however work exploring the effect of IL-6 antagonists is needed. The use of a double-blind, placebo-control animal design comparing mood alterations of a control group, a group with induced inflammation and an experimental group with induced inflammation and administration of an IL-6 antagonist would make it possible to examine the extent to which inflammation still had an affect on mood behaviour when production of IL-6 had been restricted.

#### **5.4.6 Health Consequences of Cytokine-Induced Mood Alteration**

Studies of the effects of cytokines on mood have implications for understanding the role of depression and other negative mood states in physical illness. One of the most common diseases in Britain is heart disease. Research on the relationship between depression and heart disease has evolved along two parallel lines over the last 15 years. One has examined whether depression is a risk factor for incidence of coronary heart disease (Frasure-Smith & Lesperance, 2005). The other path has examined whether depression is a risk factor for cardiovascular morbidity and mortality in the context of established coronary heart disease. Two recent meta-analyses comparing over 20 prospective studies each suggest that post-myocardial infarction depression is associated with a two-to-three fold risk of impaired cardiovascular function (Van Melle et al, 2004; Barth et al, 2004). Depressed mood has also been associated prospectively with the development of coronary artery disease, and with poor prognosis in patients with acute coronary syndromes (Hemingway et al, 2003; Lett et al, 2004). Furthermore, depression and other psychosocial factors are related to vascular inflammatory

processes implicated in atherogenesis; these include endothelial dysfunction and elevated levels of inflammatory cytokines (Steptoe & Brydon, 2005; Broadley et al, 2002). It is conceivable that inflammatory responses related to low grade infection stimulate negative moods and cardiovascular pathology, rather than mediating the relationship between the two processes. It is believed that at early stages of atherosclerosis, proinflammatory cytokines may promote further coronary atherosclerosis by enhancing macrophage and lipid deposition processes, thus promoting gradual coronary heart disease progression; while at advanced stages, low-grade inflammation can reduce plaque stability, and subsequently lead to acute coronary syndromes (Kop & Gottdiener, 2005). Elevated cytokine levels are also associated with multiple sclerosis, rheumatoid arthritis, autoimmune disease, stroke, brain trauma and Alzheimer's disease (McGeer & McGeer, 1995). Depression is highly prevalent in all these conditions, and may be in part a consequence of inflammatory responses. Further knowledge of the biological pathways linking depressed mood with disease might stimulate the development of psychopharmacological approaches that target the negative psychological effects of cytokine activation (Hayley & Anisman, 2005). In this respect, it is intriguing that aspirin prevents the endothelial dysfunction stimulated by typhoid vaccination; the effects on mood are not known (Kharbanda et al, 2002). Future directions on the role of depression and immune system involvement in cardiovascular disease is needed to, first, clarify the clinical importance of sub-syndrome depressive symptoms and sickness behaviour; second, identify the role of health behaviours and comorbid medical conditions; and third, investigate the potential relevance of monitoring immune system parameters in behavioural intervention studies.

### 5.4.7 Limitations and Conclusions

The limitations of this study should be acknowledged. The investigation was carried out with a small sample of healthy male university students, and results may not generalise to other populations. IL-6 responses to vaccination were also relatively small in comparison with those described in other investigations. Similarly, mood effects and cytokine response were not related to stress or cortisol; while the bidirectional relationship suggested between mood and IL-6 was only demonstrated on one type of vaccine. Replication of the study using different vaccinations (e.g. hepatitis B) and a larger more diverse sample is needed. Nevertheless, the results confirm that the mild transient inflammatory state induced by *salmonella typhi* vaccination has negative mood effects and that these were independent of febrile responses or physical symptoms. Vaccination may therefore be a useful model for understanding the effects of inflammation on psychological wellbeing.

The theory of allostatic load suggests that cumulative strain on the body brought about by persistent physiological activation may, over time have detrimental health effects. This theory fails however to take into account the dual-relationship that occurs between physiological activation, stimulation of complex central nervous system function, depressed mood and subsequent poor health. By employing a double-blind, placebo-controlled intervention design, this study was able to single-out the causal relationship between cytokine activation and mood in the absence of illness, thus confirming that inflammatory mechanisms are not only affected by psychological processes, but may go on to influence psychological processes as well. By extending the theory of allostatic load it is possible to conclude that sickness behaviour is initially an adaptive survival trait, but that, excessive or prolonged physiological activation can lead to impaired mood and health.



**Table 5.1: Sample Characteristics and Baseline Measures (means  $\pm$  sd)**

	<b>Vaccine (n =20)</b>	<b>Placebo (n =10)</b>
Age (years)	22 ( $\pm$ 3.1)	23 ( $\pm$ 4.3)
Systolic Blood Pressure (mmHg)	132.0 ( $\pm$ 12.1)	139.8 ( $\pm$ 18.1)
Diastolic Blood Pressure (mmHg)	73.9 ( $\pm$ 8.4)	75.3 ( $\pm$ 8.3)
Heart Rate (bpm)	72.0 ( $\pm$ 11.0)	73.3 ( $\pm$ 9.3)
Body Temperature ( $^{\circ}$ C)	36.2 ( $\pm$ .47)	36.4 ( $\pm$ .30)
IL-6 (pg/ml)	.77 ( $\pm$ .55)	.54 ( $\pm$ .41)
IL-1Ra (ng/ml)	650.22 ( $\pm$ 449.8)	811.15 ( $\pm$ 690.0)
TNF $\alpha$ (pg/ml)	2.55 ( $\pm$ .97)	2.5 ( $\pm$ .94)
Cortisol (nmol/l)	5.56 ( $\pm$ 3.29)	7.05 ( $\pm$ 6.05)
Symptoms	.70 ( $\pm$ 1.3)	.20 ( $\pm$ .42)
Overall Mood	-2.0 ( $\pm$ 8.88)	-1.6 ( $\pm$ 7.29)
Negative Mood Score	13.7 ( $\pm$ 8.29)	12.1 ( $\pm$ 6.19)
Positive Mood Score	11.7 ( $\pm$ 4.0)	10.5 ( $\pm$ 3.3)

**Table 5.2: Post-Injection Mood Change Scores (means  $\pm$  sd)**

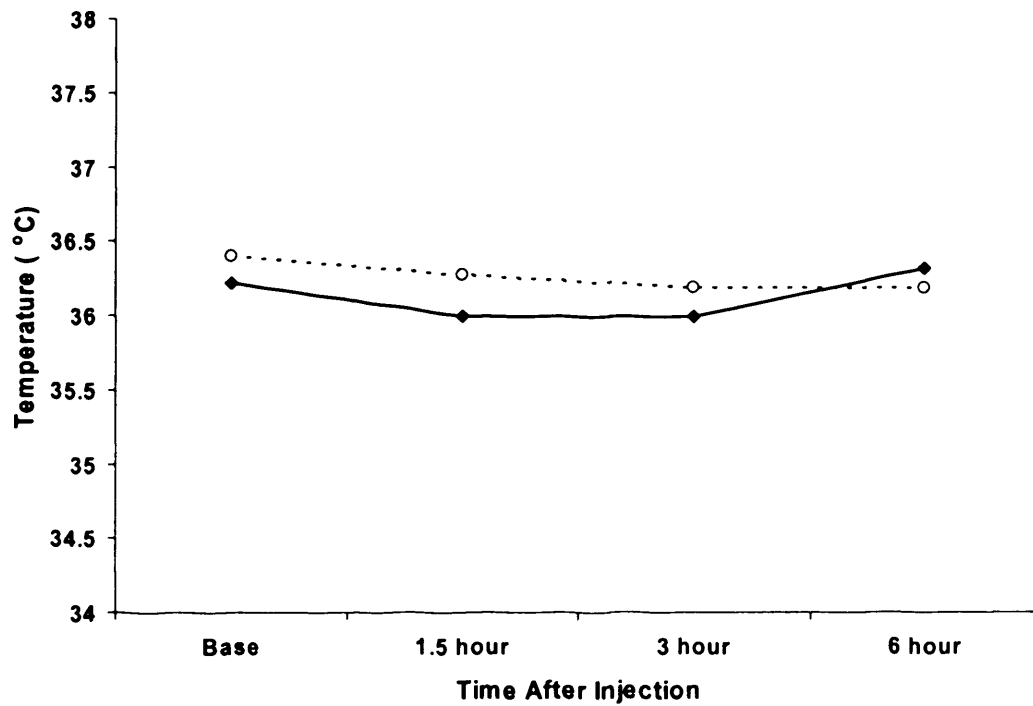
	<b>Vaccine</b>	<b>Placebo</b>
Total Mood Change Score	-.50 $\pm$ 8.9	4.8 $\pm$ 4.0
Negative Mood Change Score	-.90 $\pm$ 7.1	-4.7 $\pm$ 2.3
Positive Mood Change Score	-1.4 $\pm$ 3.6	.10 $\pm$ 3.6

**Table 5.3: Cytokine Concentration Pre and 3 hours Post-Injection (means  $\pm$  sd)**

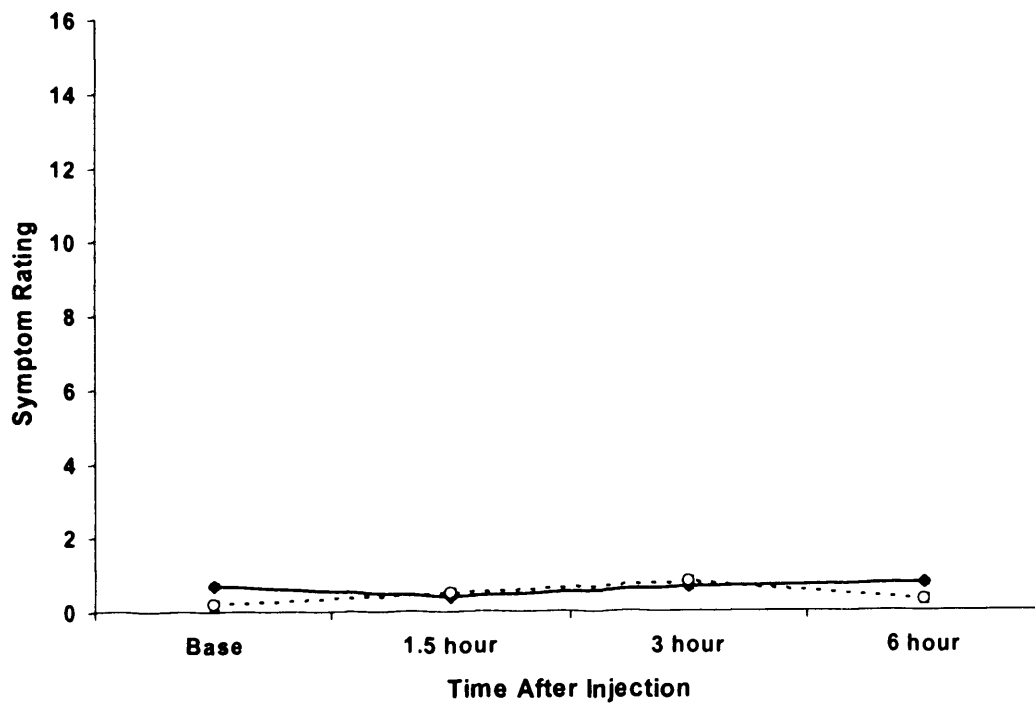
		<b>Baseline</b>	<b>3 hours</b>
IL-6 (pg/ml)	Vaccine	.77 ( $\pm$ .55)	1.59 ( $\pm$ .81)
	Placebo	.54 ( $\pm$ .41)	.53 ( $\pm$ .30)
IL-1Ra (ng/ml)	Vaccine	650.22 ( $\pm$ 449.8)	682.92 ( $\pm$ 382.55)
	Placebo	811.15 ( $\pm$ 690.0)	580.71 ( $\pm$ 366.79)
TNF $\alpha$ (pg/ml)	Vaccine	2.55 ( $\pm$ .97)	2.48 ( $\pm$ .87)
	Placebo	2.5 ( $\pm$ .94)	2.36 ( $\pm$ .69)

**Table 5.4: Post-Injection Cortisol Concentrations (nmol/l) (means  $\pm$  sd)**

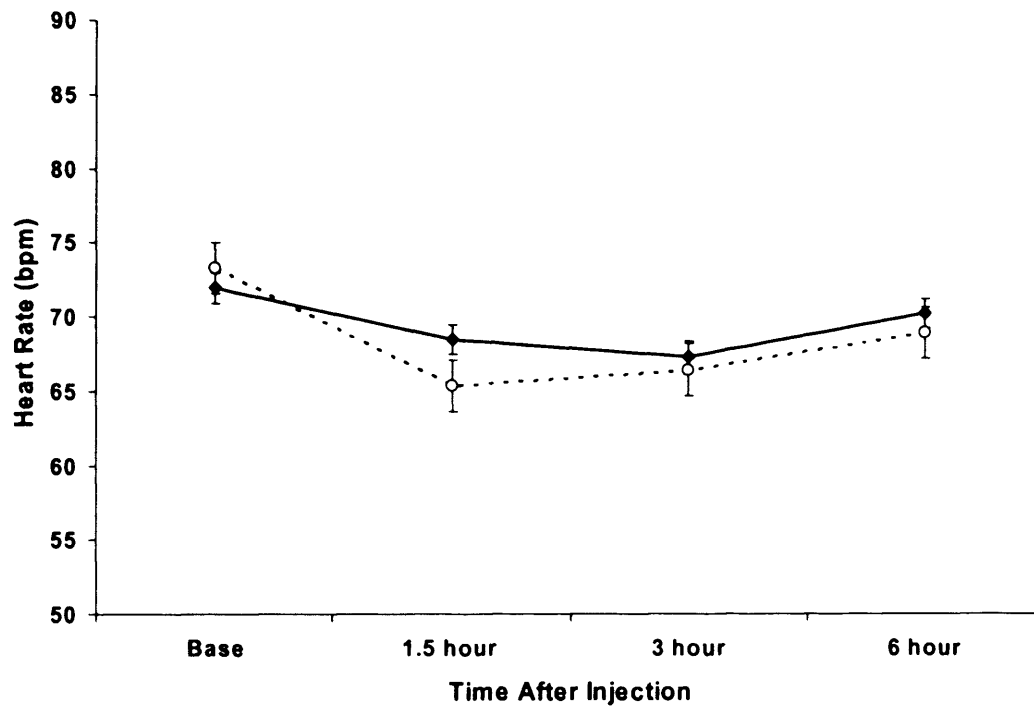
	<b>Vaccine</b>	<b>Placebo</b>
Cortisol Baseline	5.48 $\pm$ 3.2	7.05 $\pm$ 6.0
Cortisol 1.5 h Post-Injection	2.89 $\pm$ .93	2.97 $\pm$ 1.0
Cortisol 3 h Post-Injection	2.63 $\pm$ 1.7	1.69 $\pm$ .77
Cortisol 6 h Post-injection	1.75 $\pm$ .86	2.15 $\pm$ 1.05



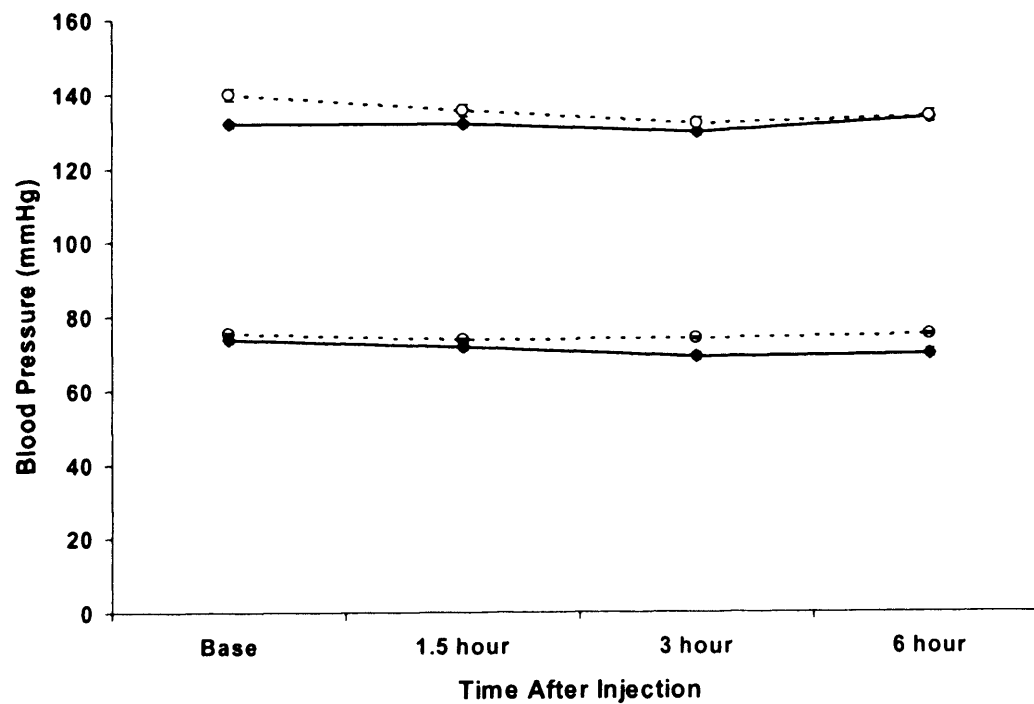
**Figure 5.1a: Body Temperature for Vaccine (solid line) and Placebo Group (dashed line) at Baseline and Post-Injection**



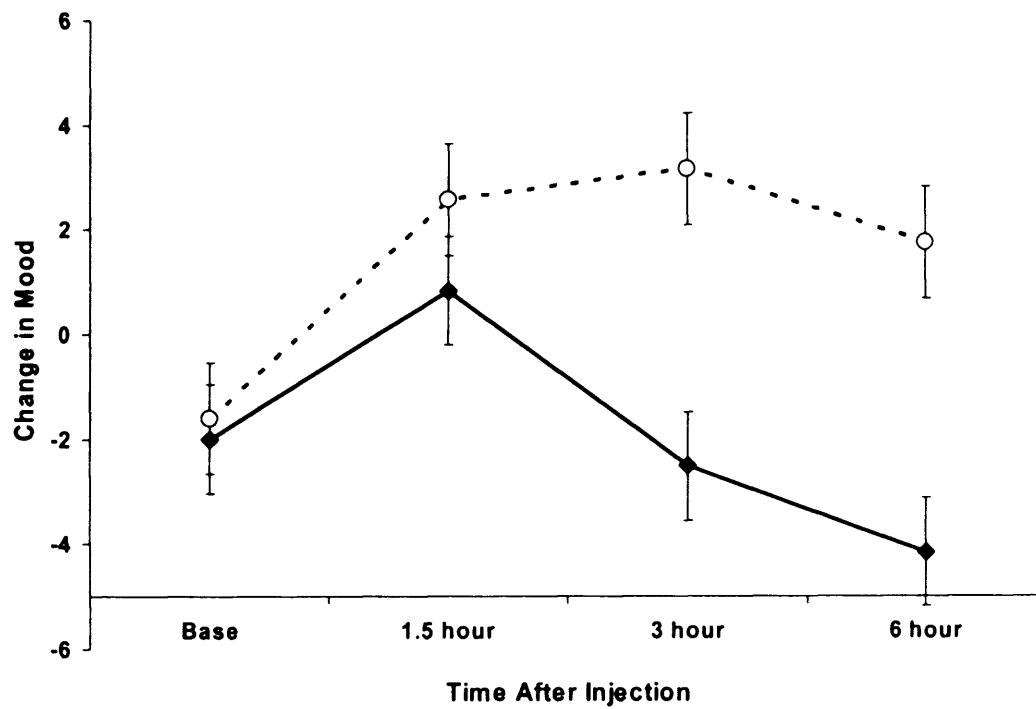
**Figure 5.1b: Symptom Rating for Vaccine (solid line) and Placebo Group (dashed line) at Baseline and Post-Injection**



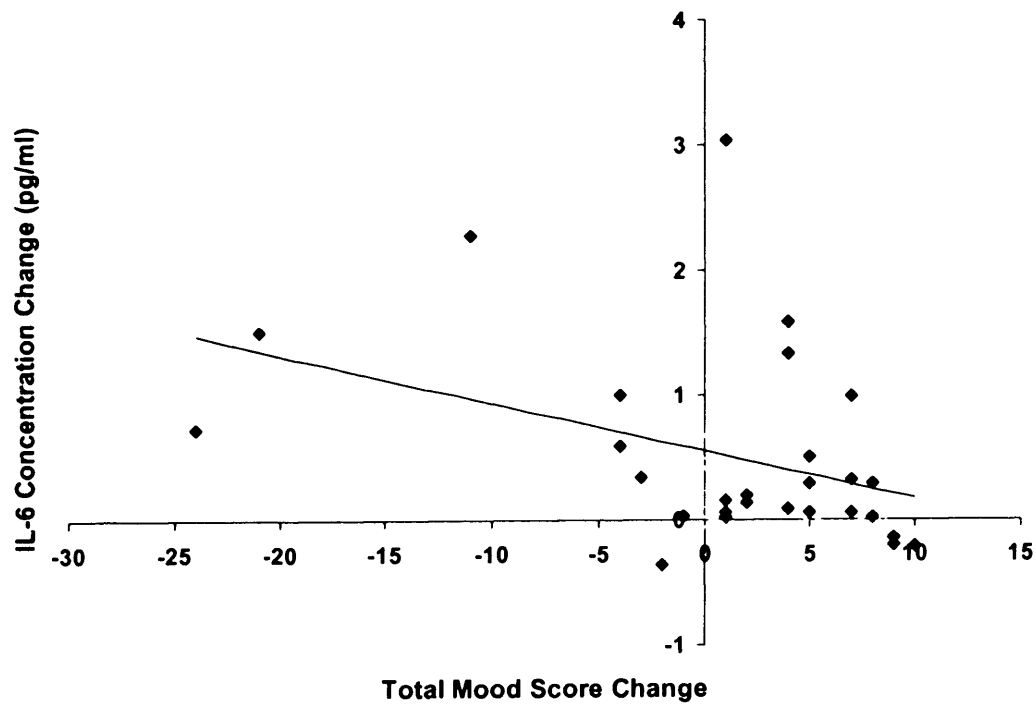
**Figure 5.1c: Heart Rate for Vaccine (solid line) and Placebo Group (dashed line) at Baseline and Post-Injection**



**Figure 5.1d: Systolic (upper lines) and Diastolic (lower lines) Blood Pressure for Vaccine (solid line) and Placebo Group (dashed line) at Baseline and Post-Injection**



**Figure 5.2: Mood Scores for Vaccine (solid line) and Placebo Group (dashed line) at Baseline and Post-Injection. Error bars are standard error of the mean.**



**Figure 5.3: Mood and IL-6 Changes in Response to Injection 3 hours Post-Injection.**

## **Chapter 6: Final Discussion**

The thesis presented a series of four studies exploring the association between individual differences in neuroendocrine, cardiovascular and immunological stress responsivity and health. Adopting the theory of allostatic load, the dissertation aimed to examine various domains of the physiological responsivity hypothesis, with a variety of populations and health outcomes across the life course. Due to the large area that allostatic load and physiological responsivity encompasses it has been impossible to cover every aspect of this topic in detail. Instead, important individual components of the stress-health relationship were chosen for closer examination. This chapter provides an opportunity to discuss the broad nature of physiological responsivity, allostatic load and health, and to highlight how inconsistencies with previous studies, and neglected areas of current research, were selected to investigate this subject. The chapter will also consider the importance (and limitations) of using a variety of samples and methodologies to explore this topic. The contribution that the four studies have made to the overall concept of allostatic load across the life course and the contribution of their findings to individual areas of psychobiology will be emphasised.

### **6.1 Theory Review: Allostasis and Allostatic Load**

Stress, in small infrequent episodes is thought to strengthen certain physiological systems and to benefit the body (Segestrom & Miller, 2004). Although the literature presented in Chapter 1 described numerous associations between stress and ill-health, relatively little is known about how elevated physiological activation eventually becomes harmful. Physiological systems respond typically to stress by initiating an

adaptive response (reactivity), sustaining it until the stressor ceases and then eliminating the response successfully (recovery) (McEwen & Seeman, 1999). It was of interest, therefore, to understand how this single response-pattern could lead ultimately to long term health impairments. In doing so, it was felt that the theory of allostatic load would be the most appropriate model to bring together and frame the four studies of this dissertation.

The theory of allostatic load states that all physiological stress mechanisms strive to maintain equilibrium, particularly during times of increased stress. This process is termed *allostasis* and refers to the body's ability to preserve 'stability through change' (Sterling & Eyer, 1988). Under certain circumstances allostasis may be threatened, and as a result *allostatic load* is said to occur (McEwen & Stellar, 1993). For each system of the body there are both short-term adaptive actions that are protective (allostasis) and long-term effects that can be damaging (allostatic load). Consequently, allostatic load may be divided into at least four sub-types, (McEwen, 1998; McEwen & Seeman, 1999; McEwen, 2000; McEwen & Wingfield, 2003a). The first type is that too much stress in the form of repeated novel events that cause recurring elevations of stress mediators occurs over a long period of time. A second type of allostatic load involves a failure to habituate or adapt to the same stressor. This leads then to an overexposure to stress mediators because of the body's failure to eliminate the physiological stress response to a repeated event. A third type of allostatic load concerns poor recovery from stress or failure to shut off the normal stress response; included with this is the failure to display the normal trough of the diurnal cortisol pattern. Finally, the fourth type of allostatic load involves an inadequate hormonal response that allows other systems, such as the inflammatory cytokines to become overactive. All four types of allostatic load are related to long term disruption in the

stress response which is defined in terms of altered reactivity or recovery. Thus, because allostatic load centres on the notion that cumulative strain will lead to physiological dysregulation and ultimately impaired health, it was deemed appropriate as the basis for the four studies in this thesis.

### **6.1.1 Thesis Findings in Accordance with the Theory of Allostatic Load**

Study 1 was designed to investigate the association between individual differences in physiological responsivity to challenge and cognitive performance in an elderly population. Allostatic load is believed to occur as a result of repeated physiological strain over the life course. The first study was based on the notion that, when the body receives significant challenges over many years, physiological systems may cease to function effectively resulting in cognitive impairment. In accordance with the theory of allostatic load, the investigation discovered that individuals with larger blood pressure and cortisol increases to a stressful challenge performed more poorly on memory tasks than individuals who were less reactive. In addition, it was found that elderly participants whose blood pressure and heart rate recovered less effectively from the challenge demonstrated poorer memory performance. The results of this first study, in keeping with the processes of allostatic load, suggest that increased cortisol or cardiovascular function, over many years (through either continued activation or failure to terminate the stress response successfully) could impair hippocampal plasticity, or facilitate the development of atherosclerosis-related white matter lesions, which in turn have detrimental effects on memory function.

As a result of these findings, Study 2 was designed to assess one of the psychosocial factors which could lead to physiological dysregulation and impaired



health in later life. Allostatic load predicts that the amount and frequency of hardship could result in a decline in health in old age. Consistent with this theory Study 2 discovered that elderly participants with lower social status had disrupted cortisol awakening responses, known to be associated with health decline.

The first two studies suggest that a cumulative effect of physiological responsivity or dysregulation could eventually lead to allostatic load and increased pathology. The idea that allostatic load causes poor health could not be determined because of the cross-sectional designs the studies employed. Study 3 therefore adopted a family history design to assess whether disturbances in physiological activation precede, rather than follow, the development of physiological health risk. A family history design identifies young healthy participants who are most at risk of developing illness in old age. Using this design, and in accordance with the theory of allostatic load, the third study revealed that participants with a positive family history of cardiovascular disease had elevated cardiovascular and cytokine responses to stress.

The first three studies in this series explored the cumulative effect of stress perception, physiological responsivity and health. In contrast, Study 4 aimed to examine whether this relationship was strictly linear as the theory of allostatic load infers. Essentially, the theory implies that stress appraisals centred in higher level brain areas affect physiological mechanisms which if persistent over time, may be detrimental to health. Study 4 however, discovered that the same regulatory pathways directly influence higher brain function, in turn altering psychological wellbeing. The findings of a bidirectional relationship suggest an extension to the original model (this is considered further in Section 6.2.3.)

In summary, the series of four studies investigate important aspects of stress research which correspond effectively with prominent parts of the theory of allostatic

load. That is, increased physiological arousal to stress, over time can results in impaired health; this is supported by the findings from Study 1. The model also suggests that cumulative hardship or adverse life events may lead to physiological disruption; this is evident in Study 2. Finally, the theory proposes that the relationship between stress physiology and poor health is contributory in nature. This was not tested directly; nevertheless, the findings of Study 3 indicate that disturbed stress physiology is present in people at increased risk of physical health problems.

## **6.1.2 Thesis Findings in Accordance with Other Stress Models**

Although the results fit the principles of allostatic load, the suitability of other stress models needs to be discussed. There are several models of stress described in the literature. As it would be impossible to review them all, only the most prominent will be considered in relation to the findings of this thesis.

### **6.1.2.1 Stimulus Based Approaches to Stress**

The first is the stimulus based approach, or life change model. This approach asserts that any life-changes a person faces will tax resources and be detrimental to health and wellbeing. This model is typically characterised by measuring stress using check lists of life events, and as such does not specifically outline the psychobiological pathways with may mediate the relationship between stress and health (Steptoe & Ayers, 2004). In this way the principles behind this model do not seem to support the finding of studies 1 to 3 which imply the role for cardiovascular neuroendocrine and immune system disturbances in the stress-health relationship. Furthermore, this approach does not take into account individuals' differences in response to stress; another element discovered in studies 1 and 3. In sum, this particular model was considered out-dated, simplistic and unsuitable for application to this thesis.

### **6.1.2.2 Response Based Approaches to Stress**

Response based approaches have concentrated on the physiological components of the stress response. The most familiar model is the General Adaptation Theory proposed by Selye. This is similar in some ways to the theory of allostatic load. It purports that the body, in the alarm phase, first activates a fight-flight response to deal with the stressor. The body then attempts to restore homeostasis in the second phase (resistance) and, exhaustion occurs if physiological resources are over-stretched, resulting in disease or death. The fundamental difference between this model and allostatic load is that the former fails to incorporate environmental provoking responses, or the role of protective psychosocial factors (Cox & Ferguson, 1991). It provides therefore, only a partial account of the stress process and does not support the results of the second study of this thesis. Similarly, response-based approaches are often too simplistic and do not account for individual subjectivity in the perception of stress, or individual differences in the magnitude of physiological response (as seen in Studies 1 and 3). The model also fails to distinguish between pathologic effects of positive and negative events and does not explain why high social status should protect against disturbance of neuroendocrine function as found in Study 2. Empirical research into the specificity of response patterning (including the findings of Studies 1 and 3) also discredits the notion of a single stress response which is elicited by all stressors.

### **6.1.2.3 Stress-Diathesis Approach**

This stress-diathesis model suggests that particular individuals may have weaknesses in one or more organ system. Specific illness then results from stress experienced by people with a genetic predisposition toward that illness. Again, this approach is viewed as simplistic as it fails to account for the specificity state of the initial stress response (Bartlett, 1998). That is, different types of stress can result in

particular types of response which lead to certain disease outcomes (as outlined by Studies 1 and 3). Furthermore, this model relies too heavily on genetic predisposition and fails to account for the range of effects the environment may have on physiological responsivity and health (as outlined in Study 2). In all, I would argue that this model does not address a number of important factors and is not suitable to represent the studies in this thesis.

#### ***6.1.2.4 Transactional Approaches to Stress***

Transactional approaches to stress emphasise individual differences in perceived stress and the importance of psychological processes, particularly cognitive appraisal. The dominant transactional model was developed by Lazarus and Folkman (1984). In this model, stress is believed to be damaging to health when demands of the environment exceed available resources. This model is advantageous in some respects because it accounts for individual differences in stress perception, and the role of environmental factors; aspects explored in this thesis and often overlooked by other models. However, there are fundamental limitations that do not make it appropriate to use in the research presented here. Primarily, the theory has been criticised because it has directed too much attention to the appraisal aspects of the stress process (Hobfoll, 2001). The model also mentions little about the variety of psychobiological pathways to disease, including the differences between causal and facilitatory processes, and the factors influencing the course of chronic disorder. I would argue, therefore, that it is inappropriate as a framework for a thesis concentrating largely on the psychobiological pathways between stress and health. In addition, the model is in danger of being untestable, because it is unfalsifiable, given that almost any empirical results concerning the effect (or lack of effect) of stress on disease may be explained by proposing a particular combination of demands, resources and vulnerabilities. Lastly, this approach

is based on the notion that a few single highly stressful events will lead to health impairments; this is again contrary to the findings of study 2 which suggest that an increased number of daily hassles in lower social status individuals could accumulate to cause dysregulation of psychobiological processes.

### **6.1.3 Section Summary**

In summary, the majority of the other stress models reviewed fail to address a number of factors fundamental to the research presented in this thesis. These include first, a the lack of consideration of individual differences in perception of stress and the physiological stress response itself; second, limited consideration of environmental factors and third, either too much or too little emphasis on the actual physiological mechanisms mediating the stress-health relationship. A further criticism is the failure to mention the time scale of the relationship between physiological activation and disease onset. The first three studies presented support the current assumption in the literature that repeated physiological activation (as opposed to single, isolated stressful events) will accumulate and eventually lead to ill-health. It is believed therefore, that allostatic load is the only model at present to satisfactorily address this important issue. In comparison to other approaches, the theory of allostatic load forces a broader opinion of the stress-health relationship, as it successfully outlines the importance of the initial protective mechanisms and longer term damaging consequences, while also taking into account the effects of individual variability and environmental factors (Schulkin, 2003).

### **6.1.4 Thesis Findings in Relation to Other Areas of Psychobiological Stress Research**

In support of the theory of allostatic load, the first three studies presented in this thesis highlight the association between neuroendocrine, cardiovascular and

immunological responsivity and health impairment. In addition to this, the studies also include other important findings, each relating to particular areas of psychobiological research.

#### ***6.1.4.1 Specific Findings from Study 1***

Study 1 highlighted the association between neuroendocrine and cardiovascular responsivity and impaired memory function in an elderly population. This cross-sectional design not only indicated a link between cumulative responsivity across the life course and poor cognitive health, but also covered more specific areas currently neglected in the literature. I attempted in this study to ensure that some of the methodological limitations of previous research were overcome. The investigation therefore included the comparison of gender differences in an elderly, community dwelling sample, while controlling statistically for possible confounds including age, education, medication use, and prevalence of chronic illness. A review of the literature indicated the majority of research in this area has focussed on cortisol responsivity and cognitive ability. Study 1 therefore investigated the associations between blood pressure reactivity, heart rate reactivity and memory performance as well. I believe that this is the first time individual rates of heart rate and blood pressure recovery have been positively associated with memory performance in an elderly sample. The investigation of both neuroendocrine and cardiovascular systems on memory impairment has not been studied in unison before. Lastly, I would argue that until now the mediating role of task appraisals on physiological and performance levels has not been fully addressed.

The results of Study 1 demonstrate that activation of the HPA axis (marked by cortisol activity) and cardiovascular systems (marked by poor blood pressure and heart rate recovery) in certain individuals during a challenging event is significantly associated with poor declarative memory performance. The findings have therefore, not

only added to the understanding of the stress process and allostatic load as a whole, but may also increase understanding and treatment options for a growing population of elderly individuals who are vulnerable to the effects of memory impairment in old age.

#### **6.1.4.2 *Specific Findings from Study 2***

Using a naturalist setting, Study 2 reported an association between cumulative strain (marked by low social status) and neuroendocrine dysfunction. Besides providing evidence for the theory of allostatic load, the results from Study 2 advances research on the cortisol response to waking. The study explored the issue of compliance to salivary cortisol sampling, which may provide greater control for future methodologies examining cortisol awakening response, particularly in an elderly sample. Furthermore, Study 2 endeavoured to compare traditional measures of social status (i.e. education and finance) with subjectively rated social position. This provided two important research developments in this specific field. First, it is believed that this is the first time that evidence of cortisol dysfunction and social status in a non-working *and* older sample has been presented. Second, the results also provide evidence that the use of subjective social ratings may be more advantageous when assessing an elderly sample, particularly those that include older women.

#### **6.1.4.3 *Specific Findings from Study 3***

Due to the cross-sectional nature of their design, studies 1 and 2 could not address the issue of cause and effect between physiological reactivity and health. Using a family history design in a ‘quasi-experimental’ laboratory setting, Study 3 discovered that participants with a family or genetic predisposition to future cardiovascular disease demonstrated elevated inflammatory and cardiovascular responses to stress. These results not only provide evidence which suggests a contributory role for stress

responsivity and disease, but also adds to research specifically applying a family history design. For example, the design of this study took added care to ensure that limitations of previous literature were addressed. This was achieved by employing a more reliable classification of positive risk which assessed the health status of two generations of the participant's family. In addition, the study extended previous research which typically concentrates on hypertension risk, to assess the risk of future cardiovascular disease and obesity development. Results indicate that young, normotensive individuals with a positive history of cardiovascular disease had increased cardiovascular and immunological responsivity to stress. These results were independent of participant's own weight and smoking status. It is believed that this is the first time that risk factors for these disorders have been considered in combination with individual stress responsivity. These findings are important in health research as they help identify individuals most at risk of cardiovascular disease, thus providing an opportunity for early intervention and improved control of hypertension, diabetes, obesity and heart disease.

#### **6.1.4.4 *Specific Findings from Study 4***

Using a placebo-control double-blind intervention, Study 4 highlighted that the relationship between physiological reactivity and psychological health is bidirectional in nature. By providing a wider perspective to the overall construct of strain, physiological processes and health the results suggest an extension to the theory of allostatic load. The findings from this last study also have important implications for research on mood, inflammation and disease. For example, the study successfully demonstrated, for the first time, that administration of *salmonella typhi* vaccine could be used as a mild model of inflammation from which to predict mood alterations and cytokine production in the absence of sickness behaviour.



## **6.2 Limitations of the Thesis Studies and Current Stress**

### **Research in General**

Although several important findings emerged from the diverse series of studies presented a number of limitations need to be considered. These take several forms, but can be divided broadly into three main areas. First, problems pertaining to interpretations of the studies' findings; second, problems relating to stress research in general; and third, problems associated with the theory of allostatic load.

#### **6.2.1 Interpretation of Study Findings**

The interpretations of findings presented in the four studies are based on three general assumptions relevant to the stress responsivity hypothesis in general. The validity of these assumptions needs to be explored.

First, conclusions drawn from Studies 1, 2 and 3 rely on the assumption that stress responsivity is a stable individual characteristic (i.e. those termed as 'high reactors' or 'poor recoverers'). Interpretation of these studies suggests that over time, these individuals will react consistently to stressful situations increasing their vulnerability to disease. Recent reviews have reported that for a variety of individual tasks, differences in cardiovascular responsivity are consistent over time (Kamarck & Lovallo, 2003; Treiber et al, 2003). For example, test-retest correlations for heart rate reactivity have ranged from 0.32 to 0.91 (Manuck et al, 1993); while cardiovascular recovery has proved relatively stable over a three year period (Rutledge et al, 2000). Based on this literature, the interpretations of the thesis findings, which suggest certain

people may be more vulnerable to disease because of their tendency to react strongly to stressors over time, seem justified.

Studies 1 and 3 employed laboratory stressors in order to assess the health consequences of individual stress responsivity. The second assumption on which interpretation of the studies findings is based, is that the physiological responses induced by these laboratory tasks will generalise to stressors encountered in everyday life. If laboratory responsivity does not generalise to real-world responses then responsivity studies in a laboratory situation cannot provide valid information about the role of stress and health. Some studies have found a significant association between laboratory task and real life stress responsivity (e.g. Pollack, 1994); however, in general, results are less definitive.

Several reviews have examined the degree to which physiological reactivity to laboratory stressors predict the response to stressors in the natural environment. For example, Pickering and Gerin (1990) examined a dozen studies and found little evidence of generalisability, while Manuck et al (1990) noted that generalisability of laboratory cardiovascular responsivity was limited by reliability of reactivity testing. In contrast, Turner et al (1994) identified 32 studies that presented moderate laboratory-to-life generalisability. It was concluded that although these associations were not strong at present, modifications to laboratory protocol may improve generalisability (Schwartz et al, 2003; Kamarck & Lovallo, 2003). These include the use of social stressors and aggregation across tasks to account for person-by situation effects. In addition, the examination of recovery responses may allow better prediction of variability in real-life responding. For this reason, Studies 1 and 3 used more than one task and investigated recovery as well as reactivity.

Lastly, and perhaps most importantly, interpretation of the studies' findings are based on the assumption that a causal relationship exists between stress responsivity and health. However, because of obvious time constraints, Studies 1, 2 and 3 employed cross-sectional designs. As a result, one can only speculate that increased neuroendocrine and cardiovascular evidence is a contributory factor towards cognitive decline in old age, and that increased cardiovascular and immunological reactivity will definitely predict future incidence of cardiovascular disease.

With regard to the relationship between cardiovascular responsivity and cardiovascular disease over time, Treiber et al (2003) provide a comprehensive review of the longitudinal literature to date. The review noted that three studies revealed an association between the cold-pressor test and future incidence of hypertension up to 30 years later (Wood et al, 1984; Menkes et al, 1989; Kasagi et al, 1995). Several studies have examined adults over a shorter period of time and have also found that stress in the laboratory predicted subsequent hypertension incidence (e.g. Falkner et al, 1981; Borghi et al, 1986; Matthews et al, 1993; Everson et al, 1996; Markovitz et al, 1998). In addition to these positive findings, there are a number of prospective studies in adults that have not found strong evidence of the unique predictive value of stress responsivity (e.g. Carroll et al, 1995; Brody et al, 1996). Despite this, Treiber et al (2003) conclude that there is reasonable evidence to suggest that cardiovascular reactivity can predict the development of cardiovascular disease. However, much more information is needed concerning the potential moderating and confounding variables of these relationships before they become clinically useful.

Similarly, although counter-evidence does exist (e.g. MacLulich et al, 2005), the results of some longitudinal prospective studies have indicated that cortisol responsivity may be causally associated with hippocampal damage and memory loss

(Lupien et al, 1994; Lupien et al, 1998) In addition, cumulative strain from low social status may stimulate physiological disturbances that are in turn related to morbidity and mortality (Lynch et al, 1997). The results of these reports suggest that over time, increased physiological reactivity can in certain individuals result in ill-health. The conclusions, speculating a causal relationship in Studies 1, 2 and 3 appear, therefore, to be justified.

### **6.2.2 Limitations of Current Stress Research**

Besides the potential problems relating to the interpretation of the findings from Studies 1, 2 and 3, there are certain areas neglected within the thesis and psychobiological stress field which merit brief discussion.

Psychobiological stress research generally investigates the magnitude of the stress responses that occur at the time at which the stressor is present. Research of this type often neglects the assessment of the frequency and duration of the responses present both before and after the stressor has been administered. That is, studies often fail to examine the speed and degree of recovery from a stressor, or responses in anticipation to a forthcoming stressor. Failure to account for these factors may mean that reactivity research is limited in its ability to reflect the multidimensional nature of real life stress responding.

The study of recovery from a stressor is known to provide additional information to that obtained by assessing stress reactivity alone (Linden et al, 1997; Linden et al, 2003), because of this, the two laboratory-based studies in this thesis attempted to examine post-task recovery. Interesting independent results were discovered for cardiovascular recovery in association with risk of cognitive decline and cardiovascular disease. Both studies were constrained by cost and time issues typical of most studies in this area. As a result it was not possible to assess cortisol recovery or cytokine

recovery in response to the stressor. Although recovery in these variables is prominent in the theory of allostatic load, very few studies to date have been able to assess this (Seeman & Robbins, 1994). This may be due to the fact that, at present, there is no universally accepted method for measuring recovery. Reports also suggest that due to methodological restrictions test-retest reliability is often poor (Christenfeld et al, 2000); while few studies have examined the generalisability of laboratory recovery in the natural environment (Rutledge et al, 2000). There is a need therefore, for future research to make a conscious effort to investigate these factors further.

In addition, there are even fewer studies which attempt to examine anticipatory responses. This is similar to the old problem of achieving a valid baseline; however research is now beginning to investigate what factors related to anticipatory stress, may be influencing actual stress response. In a recent review, Brosschot and Thayer (2004) reported that the tendency for some individuals to worry prior to a stressor may be as potent as their responses to the task itself. Failure to account for the effects of anticipatory response may have affected the findings of Studies 1 and 3. For example, during these studies it was found that systolic blood pressure (in Study 1) and cortisol (in Study 3) did not rise substantially from baseline. It could be inferred from this that the tasks were not adequately stressful; however, it should also be considered that for a number of individuals, anticipatory stress may have been as great as the response they experienced during the stressor. For these individuals the habituation period given at the beginning of the procedure may not have been adequate to overcome this. As a result it may have been advantageous to try and achieve a baseline on a separate occasion (for example a different day). Alternatively, using a post-task value towards the end of the study period may have been more accurate of resting levels. Both these alternatives have their own disadvantages, for example, there are practicalities of

gaining a large enough sample to take part in testing on two separate occasions and also, as found in Study 3, participants often become very restless towards the end of a long testing procedure. As a result it would have been beneficial in these studies to simply take into account anticipatory stress levels at the very beginning of the session using subjective ratings and physiological monitoring similar to those used throughout the study in order to determine whether a true baseline had been achieved (Sharpley, 1998).

### **6.2.3 Limitations of the Theory of Allostatic Load**

In the last few years the theory of allostatic load has come under criticism from a number of commentators. This criticism has ranged from the model being deemed too simplistic (Walsberg, 2003) to it being unnecessarily complex (Dallman, 2003). Since the studies in this thesis were framed largely by the principles of this approach, it seems appropriate to discuss the effects that these shortcomings may have on the theoretical underlay of the report.

In arguing that the approach is too simple, some authors suggest that the theory's basis in animal research means that it is not applicable to the complexities of the human environment and stress process (Björntorp, 2001). In contrast, others have contended that the model, by renaming certain aspects of the stress process (i.e. with the use of the terms allostasis and allostatic load) has done little to advance traditional models which encompass the concept of homeostasis. Dallman (2003) also contends that the model is unworkable because it will only become useful when the pathways and transmitters involved are identified; while the model often fails to account for other physiological mechanisms in the stress-disease process besides neuroendocrine reactivity (Dallman, 2003; Schulkin, 2003).

In response to these remarks, McEwen and Wingfield (2003a) argue that the theory of allostatic load is unique in the fact that it emphasises the long term cumulative

effects of the stress process across the life course and is therefore not simply redressing the concept of homeostasis. It is also contended that the model is workable. It was designed as a framework for a more integrated approach to psychobiological research, and although not all the pathways leading to disease outcome have been identified at present the theory works to guide future research so that these mechanisms can be uncovered. Defendants of the model would also agree that many other mechanisms are involved in the stress process but that to prevent unnecessary confusion cortisol was selected as an example mediator of the stress response. McEwen and Wingfield (2003a) contend that the model is applicable to many other physiological pathways and encourage investigation of them. The findings from Studies 1 and 3 indicating the role for cardiovascular and immunological stress responsivity in relation to memory decline and cardiovascular disease are therefore welcome extensions to the theory. Similarly the findings from Study 4, which revealed a bidirectional relationship between psychological perception, physiological responsivity and health, may indicate a further extension to the theory of allostatic load. For example, at present the model considers only a linear effect of stress responsivity through physiological dysregulation to health. The findings of Study 4, therefore, help to provoke future research by suggesting that health may also involve physiological mechanisms which influence psychological wellbeing.

A major obstacle in this field of research is the ability to encompass a completely holistic view of the stress health relationship. Despite the advantages that the theory of allostatic load has over other stress models, the approach has one major limitation; that is, its neglect of the cognitive appraisal process which would explain *why* certain individuals become ‘high reactors’ and are more vulnerable to stress-related disease outcomes. Neglect of cognitive appraisals is a problem which is present not

only in the studies of this report, but research in this field generally. The investigation of cognitive appraisals can be divided into two main areas: what the person is thinking prior to the stressor to elevate their physiological response, and what the person is thinking after the stressor to prolong their recovery (Brosschot et al, 2005).

The first issue concerns anticipatory cognitive appraisal. Situation appraisal is covered in great detail by the transactional theory of stress; however, the theory of allostatic load (and consequently much stress responsivity research) makes little mention of this process. Bandura's Social Cognitive Model speculates that self-efficacy and subsequent ability to cope may play a large part in one's appraisal of a potentially stressful situation. It is proposed, therefore, that individuals with lowered self-efficacy, who perceive a situation as stressful are more vulnerable to cardiovascular, neuroendocrine, immunological activation and disease progression, than ones who perceive that they have the ability to cope with an impending stressor (Bandura, 1991; Bandura, 1997). Despite this, research seldom attempts to bridge the theoretical gap between psychological and physiological responsivity. As a result, the role and importance of cognitive appraisals have received relatively little attention in stress physiology research (Ursin, 1998).

Reports do suggest however, that high cortisol responders show a personality profile characterised by low self-esteem and negative affect (Kirschbaum et al, 1995); while the relationship between cortisol response and uncontrollability is largely inconclusive (Dickerson & Kemeny, 2004). In order to advance this neglected area Gaab et al (2005) recently operationalised a questionnaire examining cognitive appraisal. Preliminary results indicate that anticipatory stress appraisal is an important determinant of cortisol stress response, explaining up to 35 % of the variance in salivary cortisol response. Knowledge about the impact of psychological processes on the



activation of physiological systems could help in the development of preventative and therapeutic interventions (Ursin & Eriksen, 2004). Further studies are clearly needed to elucidate whether retrospective assessment of stress perception is associated with other physiological pathways, and if so under what conditions.

The theory of allostatic load suggests that prolonged physiological activation once a stressor has terminated may be harmful due to the down regulation of receptors this may cause. However, the approach neglects the fact that emotional processes that sustain arousal may also be involved. A second concern is that stress responsivity research (including the studies presented in this thesis) often neglect to investigate *why* some people are ‘poor recoverers’. Although the studies in this thesis which employed stress tasks were careful to measure perceived controllability and performance, recent research has suggested that a wider psychological perspective needs to be considered. Gruenewald et al (2004) demonstrated that neuroendocrine response was elevated in participants who felt that the stressor employed threatened their ‘social self’. The Social Self Preservation Theory asserts that situations which threaten one’s social value or standing increase feelings of shame and embarrassment and decrease self-esteem, subsequently elevating ones cortisol response. Future research in this area should attempt to assess these factors in order to give a clearer picture of why some individuals might be more vulnerable to the ill effects of prolonged recovery than others.

In summary, due to the neglect of cognitive processes preceding, during and following stressful events, it may in the future be beneficial to extend the theory of allostatic load by adding certain aspects of the transactional approach or social cognitive model.

### **6.3 Implications and Directions for Future Research**

It is hoped that this thesis has highlighted that the stress-health relationship cannot be understood by examining single factors in isolation, and that the need for an integrated approach to stress responsivity research is clear. The studies presented in this thesis also demonstrated that simultaneous investigation of individual environmental, behavioural and genetic factors are crucial if understanding of the field is to advance; doing so has the potential to elucidate disease pathways, identify persons at risk of disease, and allow interventions to be tailored to individuals' predispositions and exposures.

One of the main difficulties faced by psychobiological research in the future is the investigation of whether stress responsivity is associated with disease progression or illness onset. In order to establish this relationship, the samples examined in these studies would benefit from longitudinal exploration of objective disease endpoints. For example, it would be important to ascertain whether neuroendocrine and cardiovascular dysregulation observed in Studies 1 and 2 was associated with cognitive or physical deterioration at follow-up; this could be achieved by performing clinical health assessments and obtaining neuroimaging data at regular intervals. Similarly, it would be important to assess whether the young normotensive sample with a positive family history of cardiovascular disease and adiposity investigated in Study 3 went on to gain excessive weight or develop cardiovascular disease in later life. The association between follow-up health status and physiological reactivity would also be interesting, as would the investigation of genetic differences in the high and low reactivity groups. In addition, it would be appealing to explore the relationship between stress, psychological mood and cytokine response further. This could be achieved by

examining whether the administration of a stressor at the time of vaccination would modify cytokine and mood responses.

A second difficulty encountered by psychobiological research is determination of a causal relationship between the proposed physiological mechanisms and health. Future investigation would benefit from the use of experimental (and possibly pharmacological) manipulations to confirm that interruption of proposed pathways will alter health outcome. For example, in Study 1 it was proposed that elevations in cortisol reactivity may result in hippocampal atrophy and memory impairment. Intervention studies in this sample assessing the effect of glucocorticoid antagonists or antihypertensive medication on cognitive function would be useful to investigate the causal nature of this pathway.

A related problem encountered in Studies 1, 3 and 4 was whether the neuroendocrine, cardiovascular or cytokine variables examined, were actually related to the observed health outcomes. For instance, in studies 1 and 3 it would be important to determine whether the observed changes in cortisol, heart rate and blood pressure, which were associated with memory impairment and risk of cardiovascular disease, were due directly to these variables or other elements of the HPA axis or autonomic nervous system. Pharmacological manipulation would be necessary to exact whether these associations were due to other factors, including the effects of CRH, ACTH or catecholamines on the brain. Similarly, in Study 4 it would be important to determine, with the aid of pharmacological interventions, whether the effects of cytokines on psychological mood was due solely to the influence of IL-6 or whether other cytokines were also involved.

As more is learned about the biological mechanisms which affect disease processes, it becomes increasingly important for clinicians to take stress into account in

diagnosis and treatment. Future research needs, therefore, to examine the means by which vulnerable individuals can be identified and subsequently offered effective appropriate treatment. Consequently, if we are able to develop ways, either to reduce the levels of stress to which we are exposed, or else reduce the impact of such exposure on health, then potentially the financial and social costs to society can be reduced also.

## **6.4 Conclusion**

The thesis presented a series of four separate studies investigating different aspects of the physiological stress process. The studies revealed that neuroendocrine, cardiovascular and immunological responsivity to stress each have the potential to impact upon cognitive, physical and psychological wellbeing. The findings also highlight the value of employing a diverse methodology in psychobiological research. In doing so, the thesis utilised a variety of sample ages, settings and designs in order that specific and separate aspects of the stress process could be investigated. In this was it is believed that a major strength of this work has been the breadth of the approach adopted. Finally, the use of these methodologies has enabled the identification of different psychosocial and physiological risk factors, which may be damaging to health across the life course, in the hope that advances in the theoretical understanding and clinical application of the stress-health relationship can be made.

## References

- Aartsen, M.J., Van Tilburg, T., Smits, C.H.M., Comijs, H.C., Knipscheer, K.C.P.M. (2005). Does widowhood affect memory performance of older persons? *Psychological Medicine*, 35, 217-226.
- Ackerman, K.D., Martino, M., Heyman, R., Moyna, N.M., Rabin, B.S. (1998). Stressor-induced alteration of cytokine production in multiple sclerosis patients and controls. *Psychosomatic Medicine*, 60, 484-491.
- Adler, N.E., Boyce, T., Chesney, M.A., Cohen, S. (1994a). Socioeconomic status and health: the challenge of the gradient. *American Psychologist*, 49, 15-24.
- Adler, N.E., Epel, E.S., Castellazzo, G., Ickovics, J.R. (2000). Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy white women. *Health Psychology*, 19, 586-592.
- Adler, N.E., Ostrove, J.M. (1999). Socioeconomic status and health: what we know and what we don't. *Annals of the New York Academy of Sciences*, 896, 3-15.
- Adler, N.E., Snibbe, A.C. (2003). The role of psychosocial processes in explaining the gradient between socioeconomic status and health. *Current Directions in Psychological Science*, 12, 119-123.
- Adler, P.S.J., Ditto, B. (1998). Psychophysiological effects of interviews about emotional events on offspring of hypertensives and normotensives. *International Journal of Psychophysiology*, 28, 263-271.
- Adler, P.S.J., Ditto, B., France, C., France, J. (1994b). Cardiovascular reactions to blood donation in offspring of hypertensives and normotensives. *Journal of Psychosomatic Research*, 38, 429-439.
- Affleck, G., Tennen, H., Pfeiffer, C., Fifield, J. (1987). Appraisals of control and predictability in adapting to a chronic disease. *Journal of Personality and Social Psychology*, 53, 273-279.
- Al' Absi, M., Buchanan, T.W., Marrero, A.F., Lovallo, W.R. (1999). Sex differences in pain perception and cardiovascular responses in persons with parental history for hypertension. *Pain*, 83, 331-338.
- Al' Absi, M., Petersen, K.L., Wittmers, L.E. (2000). Blood pressure but not parental history for hypertension predicts pain perception in women. *Pain*, 88, 61-68.
- Al' Absi, M., Hugdahl, K., Lovallo, W.R. (2002). Adrenocortical stress responses and altered working memory performance. *Psychophysiology*, 39, 95-99.
- Al' Absi, M., Lovallo, W.R., McKey, B., Sung, B.H., Whitsett, T., Wilson, M.F. (1998). Hypothalamic-pituitary-adrenocortical response to psychological stress and caffeine in men at high and low risk for hypertension. *Psychosomatic Medicine*, 60, 521-527.
- Albert, M., Savage, C.R., Blazer, D., Jones, K., Berkman, L.F., Seeman, T.E., Rowe, J.W. (1995). Predictors of cognitive change in older persons: MacArthur studies of successful aging. *Psychology and Aging*, 10, 578-589.

- Anderson, N.B., Armstead, C.A. (1995). Toward understanding the association of socioeconomic status and health: a new challenge for the biopsychosocial approach. *Psychosomatic Medicine*, 57, 213-225.
- Anderson, N.B., Lane, J.D., Taguchi, F., Williams, R.B.Jr. (1989). Patterns of cardiovascular response to stress as a function of race and parental hypertension in men. *Health Psychology*, 8, 525-540.
- Andresdottir, M., Sigurdsson, G., Sigvaldason, H., Gugnason, H. (2003). Family History of Myocardial infarction increases the risk of coronary heart disease, independent of other risk factors. *Evidence Based Cardiovascular Medicine*, 7, 51-52.
- Andrew, R., Phillips, D.I.W., Walker, B.R. (1998). Obesity and gender influence cortisol secretion and metabolism in men. *Journal of Clinical Endocrinology and Metabolism*, 83, 1806-1809.
- Angeli, A., Masera, R.G., Sartori, M.L., Fortunati, N., Racca, S., Dovio, A., Staurenghi, A., Frairia, R. (1999). Modulation by cytokines of glucocorticoid action. *Annals of the New York Academy of Sciences*, 876, 210-220.
- Anisman, H., Merali, Z. (1999). Anhedonic and anxiogenic effects of cytokine exposure. *Advances in Experimental and Medical Biology*, 461, 199-233.
- Arbuckle, T.Y., Gold, D.P., Andres, D., Schwartzman, A., Chaikelson, J. (1992). The role of psychological context, age and intelligence in memory performance in older men. *Psychology and Aging*, 7, 25-36.
- Artero, S., Ritchie, K. (2003). The detection of mild cognitive impairment in the general practice setting. *Aging and Mental Health*, 7, 251-258.
- Backhaus, J., Junghanns, K., Hohagen, F. (2004). Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology*, 29, 1184-1191.
- Backs, R.W., Ryan, A.M. (1992). Multimodal measures of mental workload during dual task performance: energetic demands of cognitive processes. *Proceeding of the Human Factors Society 36th Annual Meeting, Human Factors Society, Santa Monica, CA*, 1417.
- Backs, R.W., Seljos, K.A. (1994). Metabolic and cardiorespiratory measures of mental effort: the effects of level of difficulty in a working memory task. *International Journal of Psychophysiology*, 16, 57-68.
- Ballard, M.E., Cummings, E.M., Larkin, K. (1993). Emotional and cardiovascular response to adults' angry behavior and to challenging tasks in children of hypertensive and normotensive parents. *Child Development*, 64, 500-515.
- Bandura, A. (1991). Social cognitive theory of self-regulation. *Organizational Behavior & Human Decision Processes*, 50, 248-287.
- Bandura, A. (1997). *Self-Efficacy: The Exercise of Control*. New York; Freeman Times Books - Henry Holt & Co.
- Banks, W.A., Kastin, A.J. (1991). Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sciences*, 48, 117-121.
- Banks, W.A., Kastin, A.J., Gutierrez, E.G. (1994). Penetration of interleukin-6 across the murine blood-brain barrier. *Neuroscience*, 179, 53-56.

- Barnes, V.A., Treiber, F.A., Davis, H., Kelley, T.R., Strong, W.B. (1998). Central adiposity and hemodynamic functioning at rest during stress in adolescents. *International Journal of Obesity*, 22, 1079-1083.
- Barnett, R.C., Hyde, J.S. (2001). Women, men, work, and family. An expansionist theory. *American Psychologist*, 56, 781-796.
- Bartels, M., Van Den Berg, M., Sluyer, F., Boomsma, D.I., De Geus, E.J.C. (2003). Heritability of cortisol levels: review and simultaneous analysis of twin studies. *Psychoneuroendocrinology*, 28, 121-137.
- Barth, J., Schumacher, M., Herrmann-Lingen, C. (2004). Depression as a risk factor for mortality in patients with coronary heart disease: a meta-analysis. *Psychosomatic Medicine*, 66, 802-813.
- Bartlett, D. (1998). *Stress: Perspectives and Processes*. Buckingham; Philadelphia; Open University Press.
- Baum, A., Posluszny, D.M. (1999). Health psychology: mapping biobehavioral contributions to health and illness. *Annual Review of Psychology*, 50, 137-163.
- Beckwith, B.E., Petros, T.V., Scalione, C., Nelson, J. (1986). Dose-dependent effects of hydrocortisone on memory in human males. *Physiology and Behavior*, 36, 283-286.
- Beglinger, L.J., Smith, T., Gregory, R.J. (2000). Young and elderly adults' perceptions of the Wechsler Adult Intelligence Scale-Revised. *Journal of Clinical Psychology*, 56, 821-827.
- Bengtsson, K., Orho-Melander, M., Melander, O., Lindblad, U., Ranstam, J., Rastam, L., Groop, L. (2001).  $\beta_2$ -adrenergic receptor gene variation and hypertension in subjects with type 2 diabetes. *Hypertension*, 37, 1303-1308.
- Bennett, G.G., Merritt, M.M., Wolin, K.Y. (2004). Ethnicity, education, and the cortisol response to awakening: a preliminary investigation. *Ethnicity and Health*, 9, 337-347.
- Benyamini, Y., Leventhal, H., Leventhal, E.A. (2004). Self-rated oral health as an independent predictor of self-rated general health, self-esteem and life satisfaction. *Social Science and Medicine*, 59, 1109-1116.
- Beratis, S., Katrivanou, A., Georgiou, S., Monastirli, A., Pasmatzis, E., Gourzis, P., Tsambaos, D. (2005). Major depression and risk of depressive symptomatology associated with short-term and low-dose interferon- $\alpha$  treatment. *Journal of Psychosomatic Research*, 58, 15-18.
- Berntson, G.G., Bigger, J.T., Eckberg, D.L., Grossman, P., Kaufman, P.G., Malik, M., Nagaraja, H.N., Porges, S.W., Saul, J.P., Stone, P.H., Van Der Molen, M.W. (1997). Heart rate variability: origins, methods and interpretive caveats. *Psychophysiology*, 34, 623-648.
- Besedovsky, H.O., Del Rey, A. (1996). Immune-neuro-endocrine interactions: facts and hypotheses. *Endocrine Reviews*, 17, 64-102.
- Beuschlein, F., Fassnacht, M., Klink, A., Alloloi, B., Reicke, M. (2001). ACTH- receptor expression, regulation and role in adrenocortical tumor formation. *European Journal of Endocrinology*, 144, 199-206.

- Bhagwagar, Z., Hafizi, S., Cowen, P.J. (2003). Increase in concentration of waking salivary cortisol in recovered patients with depression. *American Journal of Psychiatry*, 160, 1890-1891.
- Biasucci, L.M., Liuzzo, G., Famtuzzi, G., Caligiuri, G., Rebuzzi, A.G., Ginnetti, F., Dinarello, C.A., Maser, A. (1999). Increasing levels of interleukin (IL)-Ra during the first two days of hospitalisation in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation*, 99, 2079-2094.
- Bigger, J.T., Fleiss, J.L., Steinman, R.C., Rolnitzky, L.M., Kleiger, R.E., Rottman, J.N. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation*, 85, 164-171.
- Binder, T., Frey, B., Porenta, G., Heinz, G., Wutte, M., Kreiner, G., Gossinger, H., Schmidinger, H., Pacher, R., Weber, H. (1992). Prognostic value of heart rate variability in patients awaiting cardiac transplantation. *Pacing and Clinical Electrophysiology*, 15, 2215-2220.
- Björntorp, P. (2001). Do stress reactions cause abdominal obesity and comorbidities? *Obesity Reviews*, 2, 73-88.
- Björntorp, P., Rosmond, R. (2000). Neuroendocrine abnormalities in visceral obesity. *International Journal of Obesity*, 24, S80-S85.
- Black, P.H. (1994a). Central nervous system-immune system interactions: psychoneuroendocrinology of stress and its immune consequences. *Antimicrobial Agents and Chemotherapy*, 38, 1-6.
- Black, P.H. (1994b). Immune system-central nervous system interactions: effects and immunomodulatory consequences of immune system mediators on the brain. *Antimicrobial Agents and Chemotherapy*, 38, 7-12.
- Black, P.H., Garbutt, L.D. (2002). Stress, inflammation and cardiovascular disease. *Journal of Psychosomatic Research*, 52, 1-23.
- Blackburn, I., Whalley, L., Christie, J., Shering, A., Foggo, M., Bennie, J., Farrer, D., Watts, G., Wilson, H., Fink, G. (1987). Mood, cognition and cortisol: their temporal relationships during recovery from depressive illness. *Journal of Affective Disorders*, 13, 31-43.
- Blanchard, E.B., Cornish, P.J., Wittrock, D.A., Jaccard, J. (1990). Effects of 24-hour ambulatory blood pressure monitoring on daily activities. *Health Psychology*, 9, 647-652.
- Blennow, K., Wallin, A., Uhlemann, C., Gottfries, C.G. (1991). White matter lesions on CT in Alzheimer patients: relation to clinical symptomatology and vascular factors. *Acta Neurologica Scandinavica*, 83, 187-193.
- Bonaccorse, S., Puzella, A., Marino, V., Pasquini, M., Biondi, M., Artini, M., Almerighi, C., Levvero, M., Egyed, B., Bosmans, E., Meltzer, H.Y., Maes, M. (2001). Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. *Psychiatry Research*, 105, 45-55.
- Boomsma, D.I., De Vries, J., Orlebeke, J.F. (1989). Comparison of spot and band impedance cardiogram electrodes across different tasks. *Psychophysiology*, 26, 695-699.



- Borghi, C., Costa, F.V., Boschi, S., Mussi, A., Ambrosioni, E. (1986). Predictors of stable hypertension in young borderline subjects: a 5 year follow-up study. *Journal of Cardiovascular Pharmacology*, 8, S138-S141.
- Bosma, H., Schrijvers, C., Mackenbach, J.P. (1999). Socioeconomic inequalities in mortality and importance of perceived control: cohort study. *British Medical Journal*, 319, 1469-1470.
- Bots, M.L., Van Swieten, J.C., Breteler, M.M., De Jong, F.H., Van Gijn, J., Hofman, A., Grobbee, D.E. (1993). Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. *Lancet*, 341, 1232-1237.
- Bozoki, A., Giordani, B., Heidebrink, J.L., Berent, S., Foster, N.L. (2001). Mild cognitive impairments predict dementia in non-demented elderly patients with memory loss. *Archives of Neurology*, 58, 411-416.
- Brandtstdter, J., Rothermund, K. (1994). Self-percepts of control in middle and later adult hood: buffering losses by rescaling goals. *Psychology and Aging*, 9, 265-273.
- Brenn, T., Njolstad, I. (1998). Coronary heart disease risk factors in subjects whose brothers, sisters or husbands developed premature myocardial infarction during 12 years of follow-up. *Journal of Cardiovascular Risk*, 5, 325-330.
- Breteler, M.M., Van Swieten, J.C., Bots, M.L., Grobbee, D.E., Claus, J.J., Van Den Hout, J.H.W., Van Harkamp, F., Tanghe, H.L.J., De Jong, P.T.V.M., Van Gijn, J., Hofman, A. (1994). Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. *Neurology*, 44, 1246-1252.
- Briganti, E.M., Kerr, P.G., Shaw, J.E., Zimmet, P.Z., Atkins, R.C. (2005). Prevalence and treatment of cardiovascular disease and traditional risk factors in Australian adults with renal insufficiency. *Nephrology*, 10, 40-47.
- British Heart Foundation. (2004). Blood Pressure. *Heart Information Series Number 4*, 1-60.
- Broadley, A.J.M., Korszun, A., Jones, C.J.H., Frenneaux, M.P. (2002). Arterial endothelial function is impaired in treated depression. *Heart*, 88, 521-524.
- Broderick, J.E., Arnold, D., Kudielka, B.M., Kirschbaum, C. (2004). Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology*, 29, 650.
- Brody, S., Veit, R., Rau, H. (1996). Neuroticism but not cardiovascular reactivity is associated with less longitudinal blood pressure increases. *Personality and Individual Differences*, 20, 375-380.
- Brody, S., Wagner, D., Heinrichs, M., James, A., Hellhammer, D.H., Ehlert, U. (2000). Social desirability scores are associated with higher morning cortisol levels in firefighters. *Journal of Psychosomatic Research*, 49, 227-228.
- Brosschot, J.F., Pieper, S., Thayer, J.F. (2005). Expanding stress theory: prolonged activation and perseverative cognition. *Psychoneuroendocrinology*, 30, 1043-1049.
- Brosschot, J.F., Thayer, J.F. (2004). Worry, perseverative thinking and health. In Nyklicek, I., Temoshok, L.R., Vingerhoets, A.J.J.M. (Eds.). *Emotional Expression and Health: Advances in Theory, Assessment and Clinical Application*. London; Taylor Francis.

- Brown, E.S., Varghese, F.P., McEwen, B.S. (2004). Association of depression with medical illness: does cortisol play a role? *Biological Psychiatry*, 55, 1-9.
- Brydon, L., Edwards, S., Mohamed-Ali, V., Steptoe, A. (2004). Socioeconomic status and stress-induced increases in interleukin-6. *Brain Behavior and Immunity*, 18, 281-290.
- Brydon, L., Steptoe, A. (2005). Stress-induced increases in interleukin-6 and fibrinogen predict ambulatory blood pressure at 3-year follow-up. *Journal of Hypertension*, 23, 1001-1007.
- Budge, M.M., De Jager, C., Hogervorst, E., Smith, D. (2002). Total plasma homocysteine, age, systolic blood pressure and cognitive performance in older people. *Journal of the American Geriatric Society*, 50, 2014-2018.
- Bujalska, I.J., Kumar, S., Stewart, P.M. (1997). Does central obesity reflect "Cushing's disease of the omentum"? *Lancet*, 349, 1210-1213.
- Burleson, M.H., Poehlmann, K.M., Hawley, L.C., Ernst, J.M., Berntson, G.G., Malarkey, W.B., Kiecolt-Glaser, J.K., Glaser, R., Cacioppo, J.T. (2003). Neuroendocrine and cardiovascular reactivity to stress in mid-aged and older women: long term temporal consistency of individual differences. *Psychophysiology*, 40, 358-369.
- Burns, A., Zaudig, M. (2002). Mild cognitive impairment in older people. *The Lancet*, 360, 1963-1965.
- Buss, C., Wolf, O.T., Witt, J., Hellhammer, D.H. (2004). Autobiographical memory impairment following acute cortisol administration. *Psychoneuroendocrinology*, 29, 1093-1096.
- Cacciatore, F., Abete, P., Ferrara, N., Paolisso, G., Amato, L., Canonico, S., Maggi, S., Varrichio, M., Rengo, F. (1997). The role of blood pressure in cognitive impairment in an elderly population. *Journal of Hypertension*, 15, 135-142.
- Cacioppo, J.T., Malarkey, W.B., Kiecolt-Glaser, J.K., Uchino, B.N., Sgoutas-Emch, S.A., Sheridan, J.F., Berntson, G., Glaser, R. (1995). Heterogeneity in neuroendocrine and immune response to brief psychosocial stressors as a function of autonomic cardiac activation. *Psychosomatic Medicine*, 57, 154-164.
- Cao, C., Matsumura, K., Yamagata, K., Watanabe, Y. (1996). Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1 $\beta$ : a possible site of prostaglandin synthesis responsible for fever. *Brain Research*, 733, 263-272.
- Cao, C., Matsumura, K., Yamagata, K., Watanabe, Y. (1997). Involvement of cyclooxygenase-2 in LPS-induced fever and regulation of its mRNA by LPS in the rat brain. *American Journal of Physiology*, 272, R1712-R1725.
- Cannon, W.B. (1914) The emergency function of the adrenal medulla in pain and the major emotions. *American Journal of Physiology*, 33, 356-372.
- Capuron, L., Dantzer, R. (2003). Cytokines and depression: the need for a new paradigm. *Brain Behavior and Immunity*, 17, S119-S124.
- Capuron, L., Lamarque, D., Dantzer, R., Goodall, G. (1999). Attentional and mnemonic deficits associated with infectious diseases in humans. *Psychological Medicine*, 29, 291-297.

- Capuron, L., Ravaut, A., Gualde, N., Bosmans, E., Dantzer, R., Maes, M., Neveu, P.J. (2001). Association between immune activation and early depressive symptoms in cancer patients treated with interleukin-2-based therapy. *Psychoneuroendocrinology*, 26, 797-808.
- Carlson, L.E., Sherwin, B.B. (1999). Relationships among cortisol (CRT), dehydroepiandrosterone-sulphate (DHEAS), and memory in a longitudinal study of healthy elderly men and women. *Neurobiology of Aging*, 20, 315-324.
- Carroll, D., Davy Smith, G., Sheffield, D., Shipley, M.J., Marmot, M.G. (1995). Pressor reactions to psychological stress and predictions of future blood pressure: data from Whitehall II study. *British Medical Journal*, 310, 771-776.
- Carstensen, L.L., Freund, A.M. (1994). The resilience of the aging self. *Developmental Review*, 14, 81-92.
- Castanon, N., Leonard, B.E., Neveu, P.J., Yirmiya, R. (2002). Effects of antidepressants on cytokine production and actions. *Brain Behavior and Immunity*, 16, 569-574.
- Chambless, L.E., Heiss, G., Shaha, E., Earp, M.J., Toole, J. (2004). Prediction of ischemic stroke risk in the Atherosclerosis Risk in Communities Study. *American Journal of Epidemiology*, 160, 259-269.
- Checkley, S.A., Raven, P., O'Dwyer, A.-M., Segovia, M., Lightman, S., Mulligan, O., Reynolds, M. (1997). Antidepressant effects of metyrapone. *Biological Psychiatry*, 42, S220-S223.
- Chipperfield, J.G., Greenslade, L. (1997). Perceived control as a buffer in the use of health care services. *Journals of Gerontology: Series B - Psychological Sciences & Social Sciences*, 54, 146-154.
- Christenfeld, N., Glynn, L.M., Gerin, W. (2000). On the reliable assessment of cardiovascular recovery: an application of curve fitting techniques. *Psychophysiology*, 37, 543-550.
- Chrousos, G.P. (2005). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *New England Journal of Medicine*, 332, 1351-1362.
- Clow, A. (2001). The physiology of stress. In Jones, F., Bright, J. (Eds.). *Stress: Myth, Theory and Research*. London; Prentice Hall.
- Clow, A., Patel, S., Najafi, M., Evans, A., Hucklebridge, F. (1997). The cortisol response to psychological challenge is preceded by a transient rise in endogenous inhibitor of monoamine oxidase. *Life Sciences*, 61, 567-575.
- Clow, A., Thorn, E., Evans, A., Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. *Stress*, 7, 29-37.
- Cohen, S., Karamak, T. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, 24, 385-396.
- Cohen, S.A., Evans, G.W., Krantz, D.S., Stokols, D. (1980). Physiological, motivational and cognitive effects of aircraft noise on children. *American Psychologist*, 35, 231-243.
- Cohen, S.A., Glass, D.C., Singer, J.E. (1973). Apartment noise, auditory discrimination, and reading ability in children. *Journal of Experimental Social Psychology*, 9, 407-422.

- Colao, A., Pivonello, R., Spiezia, S., Faggiano, A., Ferone, D., Filippella, M., Marzullo, P., Cerbone, G., Siciliani, M., Lombardi, G. (1999). Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *Journal of Clinical Endocrinology and Metabolism*, 84, 2664-2672.
- Corwin, E.J. (2000a). Understanding cytokines part I: physiology and mechanism of action. *Biological Research for Nursing*, 2, 30-40.
- Corwin, E.J. (2000b). Understanding cytokines part II: implications for nursing research and practice. *Biological Research for Nursing*, 2, 41-48.
- Costello, N.L., Bragdon, E.E., Light, K.C., Sigurdsson, A., Bunting, S., Grewen, K., Maixner, W. (2002). Temporomandibular disorder and optimism: relationships to ischemic pain sensitivity and interleukin-6. *Pain*, 100, 99-110.
- Cox, T., Ferguson, E. (1991). Individual differences in stress and coping. In Cooper, C.L., Payne, R. (Eds.). *Personality and Stress: Individual Differences in the Stress Process*. Chichester; John Wiley.
- Crandall, C.S., Preisler, J.J., Aussprung, J. (1992). Measuring life event stress in the lives of college students: the Undergraduate Stress Questionnaire. *Journal of Behavioral Medicine*, 15, 327-662.
- Crimmins, E.M., Johnston, M., Hayward, M., Seeman, T. (2003). Age differences in allostatic load: an index of physiological dysregulation. *Experimental Gerontology*, 38, 731-734.
- Dallman, M.F. (2003). Stress by any other name.... *Hormones and Behavior*, 43, 18-20.
- Dalton, M., Cameron, A.J., Zimmet, P.Z., Shaw, J.E., Jolley, D., Dunstan, D.W., Welborn, T.A. (2003). Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *Journal of Internal Medicine*, 254, 555-563.
- Dantzer, R. (2001). Cytokine-induced sickness behavior: where do we stand? *Brain Behavior and Immunity*, 15, 7-24.
- Dantzer, R. (2004). Cytokine-induced sickness behavior: a neuroimmune response to activation of innate immunity. *European Journal of Pharmacology*, 500, 399-411.
- Dantzer, R., Wollman, E., Vitkovic, L., Yirmiya, R. (1999). Cytokines and depression: fortuitous or causative association? *Molecular Psychiatry*, 4, 328-332.
- Davey Smith, G., Hart, C., Hole, D., MacKinnon, P., Gillis, C., Watt, G., Blane, D., Hawthorne, V. (1998). Education and occupational social class: which is the more important indicator of mortality risk? *Journal of Epidemiology and Community Health*, 52, 153-160.
- Davis, M.C., Twamley, E.W., Hamilton, N.A., Swan, P.D. (1999). Body fat distribution and hemodynamic stress responses in premenstrual obese women: a preliminary study. *Health Psychology*, 18, 625-633.
- De Kloet, E.R. (1991). Brain corticosteroid receptor balance and homeostatic control. *Neuroendocrinology*, 12, 95-164.
- De Kloet, E.R., Oitzl, M.S., Joëls, M. (1999). Stress and cognition: are corticosteroids good or bag guys? *Trends in Neuroscience*, 22, 422-426.

- De Leon, M., Golomb, J., George, A.E., Convit, A., Tarshish, C., McRae, T., De Santi, S., Smith, G., Ferris, S.H., Noz, M., Rusinek, H. (1993). The radio-logic prediction of Alzheimer Disease: the atrophic hippocampal formation. *American Journal of Neuroradiology*, 14, 897-906.
- De Leon, M., McRae, T., Tsai, J.R., George, A.E., Marcus, D.L., Freedman, M., Wolf, A.P., McEwen, B.S. (1988). Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy. *Lancet*, 8607, 391-392.
- De Quervain, D.J.-F., Roozendaal, B., Nitsch, R.M., McGaugh, J.L., Hock, C. (2000). Acute cortisol administration impairs retrieval of long-term declarative memory in humans. *Nature Neuroscience*, 3, 313-314.
- De Sutter, J., De Bacquer, D., Kotseva, K., Sans, S., Pyörälä, K., Wood, D., De Backer, G. (2003). Screening of family members of patients with premature coronary heart disease: Results from the EUROASPIRE II family survey. *European Heart Journal*, 24, 249-247.
- De Visser, D.C., Van Hooft, I.M.S., Van Doornen, L.J.P., Hofman, A., Orlebeke, J.F., Grobbee, D.E. (1995). Cardiovascular response to mental stress in offspring of hypertensive parents: the Dutch Hypertension and Offspring Study. *Journal of Hypertension*, 13, 901-908.
- Dekker, J.M., Crow, R.S., Folsom, A.R., Hannan, P.J., Liao, D., Swenne, C.A., Schouten, E.G. (2000). Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: The ARIC study. *Circulation*, 102, 1239-1244.
- Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Korer, A., Schmider, J., Standhardt, H., Lammers, C.H., Heuser, I. (1997). With aging in humans the activity of the hypothalamic-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sciences*, 61, 2239-2246.
- Diamond, D.M., Bennet, D.A., Fleshner, M., Rose, G.M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal prime-burst potentiation. *Hippocampus*, 2, 421-430.
- Dibbs, Z., Kurrelmeyer, K., Kalra, D., Seta, Y., Wang, F., Bozkurt, B., Baumgarten, G., Sivasubramania, N., Mann, D.L. (1999). Cytokines in heart failure: pathogenetic mechanisms and potential treatment. *Proceedings of the National Academy of Sciences*, 111, 423-428.
- Dickerson, S.S., Kemeny, M.E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, 130, 355-391.
- Dinarello, C.A. (1999). Cytokines as endogenous pyrogens. *Journal of Infectious Disease*, 179, S294-S304.
- Dobbin, J.P., Harth, M., McCain, G.A., Martin, R.A., Cousin, K. (1991). Cytokine production and lymphocyte transformation during stress. *Brain Behavior and Immunity*, 5, 339-348.
- Domes, G., Heinrichs, M., Reichwald, U., Hautzinger, M. (2002). Hypothalamic-pituitary-adrenal axis reactivity to psychological stress and memory in middle-aged women; high responders exhibit enhanced declarative memory performance. *Psychoneuroendocrinology*, 27, 843-853.

- Dressendorfer, R.A., Kirschbaum, C., Rohde, W., Stahl, F., Strasburger, C.J. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *Journal of Steroid Biochemistry & Molecular Biology*, 43, 683-692.
- Dugue, B., Leppanen, E., Grasbeck, R., Benoit, D., Esa, L., Ralph, G. (2001). Effects of psychological stress on plasma interleukin-1 beta and 6, C-reactive protein, tumor necrosis factor alpha, anti diuretic hormone and serum cortisol. *Scandinavian Journal of Clinical and Laboratory Investigation*, 53, 555-561.
- Duncan, M.R., Berman, B. (1991). Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6. *Journal of Investigative Dermatology*, 97, 686-692.
- Dunn, A.J. (2001). Effects of cytokines and infections on brain neurochemistry. In Adler, R., Felten, D.L., Cohen, N. (Eds.). *Psychoneuroimmunology*. New York; Academic Press.
- Dustman, R.E., Emmerson, R.Y., Shearer, D.E. (1990). Aerobic fitness may contribute to CNS health: electrophysiological, visual and neurocognitive evidence. *Journal of Neurologic Rehabilitation*, 4, 241-254.
- Dyer, A.R., Persky, V., Stamer, J., Paul, O., Shekele, R.B., Berkson, D.M., Lepper, M., Schoenberger, J.A., Lindberg, H.A. (1980). Heart rate as a prognostic factor for coronary artery disease and mortality: findings in three Chicago epidemiologic studies. *American Journal of Epidemiology*, 112, 736-749.
- Earle, T.L., Linden, W., Weinberg, J. (1999). Differential effects on harassment on cardiovascular and salivary cortisol stress reactivity and recovery in men and women. *Journal of Psychosomatic Research*, 46, 125-141.
- Eckel, R.H. (1997). Obesity and heart disease: a statement for health care professionals from the nutrition committee, American Heart Association. *Circulation*, 96, 3248-3250.
- Edwards, S., Evans, P., Hucklebridge, F., Clow, A. (2001). Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology*, 26, 613-622.
- Eisenmann, J.C. (2003). Secular trends in variables associated with the metabolic syndrome of North American children and adolescents: a review and synthesis. *American Journal of Human Biology*, 15, 786-794.
- Elder, G.H., George, L.K., Shanahan, M.G. (1996). Psychological stress over the life course. In Kaplan, H. (Ed.). *Perspective on Psychological Stress*. San Diego; Academic Press.
- Elias, M.F., Wolf, P.A., D'Agostino, R.B., Cobb, J., White, L.R. (1993). Untreated blood pressure level is universally related to cognitive functioning: The Framingham Heart Study. *American Journal of Epidemiology*, 138, 353-364.
- Englund, E., Brun, A., Alling, C. (1988). White matter changes in dementia of Alzheimer's type. Biochemical and neuropathological correlates. *Brain*, 111, 1425-1439.
- Ensel, W.M. (1991). 'Important' life events and depression among older adults. *Journal of Aging and Health*, 3, 546-566.
- Epel, E.S., McEwen, B., Seeman, T., Matthews, K., Castellazzo, G., Brownell, K., Bell, J., Ickovics, J.R. (2000). Stress and body shape: stress-induced cortisol secretion is

- consistently greater among women with central fat. *Psychosomatic Medicine*, 62, 623-632.
- Ernst, S.A., Lovallo, W.R., Sausen, K.P., Wilson, M.F. (1990). Manifest hostility may affect habituation of cardiovascular reactivity in Blacks. *Behavioral Medicine*, 16, 119-124.
- Everson, S.A., Kaplan, G.A., Goldberg, D.E., Salonen, J.T. (1996). Anticipatory blood pressure response to exercise predicts future high blood pressure in middle-aged men. *Hypertension*, 27, 1059-1064.
- Fahdi, I.E., Gaddam, V., Garza, L., Romeo, F., Mehta, J.L. (2003). Inflammation, infection, and atherosclerosis. *Brain Behavior and Immunity*, 17, 238-244.
- Falkner, B., Kushner, H., Onesti, G., Angelakos, E.T. (1981). Cardiovascular characteristics in adolescents who develop essential hypertension. *Hypertension*, 3, 521-527.
- Falkner, B., Onesti, G., Angelakos, E.T., Fernandes, M., Langman, C. (1979). Cardiovascular response to mental stress in normal adolescents with hypertensive parents. Hemodynamics and mental stress in adolescents. *Hypertension*, 1, 23-30.
- Farmer, M.E., Kittner, S.J., Abbott, R.D., Wolz, M.M., Wolf, P.A., White, L.R. (1990). Longitudinally measured blood pressure, antihypertensive medication use, and cognitive performance: The Framingham Study. *Journal of Clinical Epidemiology*, 43, 475-480.
- Faucheux, B.A., Dupuis, C., Baulon, A., Lille, F., Bourlief, F. (1983). Heart rate reactivity during minor mental stress in men in their 50's and 70's. *Gerontology*, 29, 149-160.
- Federenko, I., Wüst, S., Hellhammer, D.H., Dechoux, R., Kumsta, R., Kirschbaum, C. (2004). Free cortisol awakening responses are influenced by awakening time. *Psychoneuroendocrinology*, 29, 174-184.
- Fehm-Wolfsdorf, G., Reutter, K., Zenz, H., Born, J., Fehm, H.L. (1993). Are circadian variations in taste thresholds cortisol-dependent? *Journal of Psychophysiology*, 7, 65-77.
- Feldman, P.J., Cohen, S., Lepore, S.J., Matthews, K.A., Kamarck, T.W., Marsland, A.L. (1999). Negative emotions and acute physiological responses to stress. *Annals of Behavioral Medicine*, 21, 216-222.
- Ferrucci, L., Penninx, B.W., Volpato, S., Harris, T.B., Bandeen-Roche, K., Balfour, J., Leveille, S.G., Fried, L.P. (2002). Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. *Journal of the American Geriatrics Society*, 50, 1947-1954.
- Folkman, S., Lazarus, R.S., Pimley, S., Novacek, J. (1987). Age differences in stress and coping processes. *Psychology and Aging*, 2, 171-184.
- France, C., Stewart, K.M. (1995). Parental history of hypertension and enhanced cardiovascular reactivity are associated with decreased pain ratings. *Psychophysiology*, 32, 571-578.
- Frasure-Smith, N., Lesperance, F. (2005). Reflections in depression as a cardiac risk factor. *Psychosomatic Medicine*, 67, S19-S25.
- Frazer, N.L., Larkin, K.T., Goodie, J.L. (2002). Do behavioral responses mediate or moderate the relation between cardiovascular reactivity to stress and parental history of hypertension? *Health Psychology*, 21, 244-253.

- Fredrikson, M., Matthews, K.A. (1990). Cardiovascular responses to behavioral stress and hypertension: a meta-analytic review. *Annals of Behavioral Medicine*, 12, 30-39.
- Fredrikson, M., Tuomisto, M., Bergman-Losman, B. (1991). Neuroendocrine and cardiovascular stress reactivity in middle-aged normotensive adults with parental history of cardiovascular disease. *Psychophysiology*, 28, 656-664.
- Frenneaux, M.P. (2004). Autonomic changes in patients with heart failure and in post-myocardial infarction patients. *Heart*, 90, 1248-1255.
- Fried, S.K., Ricci, M.R., Russel, C.D., LaFerrere, B. (2000). Regulation of leptin production in humans. *Journal of Nutrition*, 130, S3127-S3131.
- Gaab, J., Rohleder, N., Nater, U.M., Ehlert, U. (2005). Psychological determinants of the cortisol stress response: the role of anticipatory cognitive appraisal. *Psychoneuroendocrinology*, 30, 599-610.
- Gabay, C., Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine*, 340, 448-454.
- Galea, L.A.M., Tanapat, P., Gloud, E. (1996). Exposure to predator odor suppresses cell proliferation in the dentate gyrus of adult rats via a cholinergic mechanism. *Society for Neuroscience Abstracts*, 22, 314.
- Gerin, W., Christenfeld, N., Pieper, C., DeRafael, D.A., Su, O., Stroessner, S.J., Deich, J., Pickering, T.G. (1998). The generalizability of cardiovascular responses across settings. *Journal of Psychosomatic Research*, 44, 209-218.
- Gerin, W., Pickering, T.G. (1995). Association between delayed recovery of blood pressure after acute mental stress and parental history of hypertension. *Journal of Hypertension*, 13, 603-610.
- Gerin, W., Rosofsky, M., Pieper, C., Pickering, T.G. (1994). A test of laboratory-to-life generalisability using a controlled ambulatory procedure. *Psychosomatic Medicine*, 56, 360-368.
- Giles, L.C., Glonek, G.F.V., Luszcz, M.A., Andrews, G.R. (2005). The effect of social networks on 10-year survival in very old Australians: the Australian Longitudinal Study of Ageing. *Journal of Epidemiology and Community Health*, 59, 574-479.
- Girod, J.P., Brotman, D.J. (2004). Does altered glucocorticoid homeostasis increase cardiovascular risk? *Cardiovascular Research*, 64, 217-226.
- Glaser, R., Robles, T.F., Sheridan, J., Malarkey, W., Kiecolt-Glaser, J.K. (2003). Mild depressive symptoms are associated with amplified and prolonged inflammatory responses after influenza virus vaccination in older adults. *Archives of General Psychiatry*, 60, 1009-1014.
- Gloud, E., McEwen, B.S., Tanapat, P., Galea, L.A.M., Fuchs, E. (1997). Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *Journal of Neuroscience*, 17, 2492-2498.
- Gloud, E., Tanapat, P., McEwen, B.S., Flugge, G., Fuchs, E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys diminished by stress. *Proceedings of the National Academy of Sciences*, 95, 3168-3171.



- Glynn, L.M., Christenfeld, N., Gerin, W. (2002). The role of rumination in recovery from reactivity: cardiovascular consequences of emotional states. *Psychosomatic Medicine*, 64, 714-726.
- Glynn, R.J., Beckett, L.A., Hebert, L.E., Morris, M.C., Scherr, P.A., Evans, D.A. (1999). Current and remote blood pressure and cognitive decline. *Journal of the American Medical Association*, 281, 438-445.
- Goebel, M.U., Mills, P.J., Irwin, M., Ziegler, M.G. (2000). Interleukin-6 and tumor necrosis factor- $\alpha$  production after acute psychological stress, exercise, and infused isoproterenol: differential effects and pathways. *Psychosomatic Medicine*, 62, 591-598.
- Gold, P.W., Wong, M.L., Chrousos, G.P., Licinio, J. (1996). Stress system abnormalities in melancholic and atypical depression: molecular, pathophysiological, and therapeutic implications. *Molecular Psychiatry*, 1, 257-265.
- Goldberg, A.L., Rigney, D.R., West, B.J. (1990). Chaos and fractals in human physiology. *Scientific America*, 262, 43-49.
- Gotthardt, U., Schweiger, U., Fahrenberg, J., Lauer, C.J., Holsboer, F., Heuser, I. (1995). Cortisol, ACTH, and cardiovascular response to a cognitive change paradigm in aging and depression. *American Journal of Physiology*, 268, 865-873.
- Grassi, G., Esler, M. (1999) How to assess sympathetic activity in humans. *Journal of Hypertension*, 17, 719-734.
- Grossi, G., Perski, A., Lundberg, U., Soares, J. (2001). Associations between financial strain and the diurnal salivary cortisol secretion of long-term unemployed individuals. *Integrative Physiological and Behavioral Science*, 36, 205-219.
- Gruenewald, T.L., Kemeny, M.E., Aziz, N., Fahey, J.L. (2004). Acute treat to the self: shame, social self-esteem, and cortisol activity. *Psychosomatic Medicine*, 66, 915-924.
- Gulpinar, M.A., Yegen, B.C. (2004). The physiology of learning and memory: the role of peptides and stress. *Current Protein and Peptide Science*, 5, 457-473.
- Gump, B.B., Matthews, K.A., Raikkonen, K. (1999). Modelling relationships among socioeconomic status, hostility, cardiovascular reactivity, and left ventricular mass in African American and White children. *Health Psychology*, 18, 140-150.
- Haack, M., Hinze-Selch, D., Fenzel, T., Kraus, T., Kuh, M., Schuld, A., Pollmächer, T. (1999). Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. *Journal of Psychiatric Research*, 33, 407-418.
- Hajdu, M.A., Heistad, D.D., Siems, J.E., Baumbach, G.L. (1990). The effects of aging of mechanics and composition of cerebral arterioles in rats. *Circulation Research*, 66, 1747-1754.
- Hansen, A.L., Johnsen, B.H., Thayer, J.F. (2003). Vagal influence on working memory and attention. *International Journal of Psychophysiology*, 48, 263-274.
- Harris, T.B., Ferrucci, L., Tracy, R.P., Corti, M.C., Wacholder, S., Ettinger, W.H.Jr., Heimovitz, H., Cohen, H.J., Wallace, R. (1999). Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *American Journal of Medicine*, 106, 506-512.

- Hart, B.L. (1988). Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioral Reviews*, 12, 123-137.
- Hartmann, A., Veldhuis, J.D., Deuschle, M., Standhardt, H., Heuser, I. (1997). Twenty-four hour cortisol release profiles in patients with Alzheimer's and Parkinson's disease compared to normal controls: ultradian secretory pulsatility and diurnal variation. *Neurobiology of Aging*, 18, 285-289.
- Hawkley, L.C., Burleson, M.H., Poehlmann, K.M., Berntson, G.G., Malarkey, W.B., Cacioppo, J.T. (2001). Cardiovascular and endocrine reactivity in older females: intertask consistency. *Psychophysiology*, 38, 863-872.
- Hayakawa, T., Nagai, Y., Kahara, T., Yamashita, H., Takamura, T., Abe, T., Nomura, G., Kobayashi, K. (2000). Gln27Glu and Arg16Gly polymorphisms of the beta2-adrenergic receptor gene are not associated with obesity in Japanese men. *Metabolism*, 49, 1215-1218.
- Hayley, S., Anisman, H. (2005). Multiple mechanisms of cytokine action in neurodegenerative and psychiatric states: Neurochemical and molecular substrates. *Current Pharmaceutical Design*, 11, 947-962.
- Hayney, M.S., Love, G.D., Buck, J.M., Ryff, C.D., Singer, B., Muller, D. (2003). The association between psychosocial factors and vaccine-induced cytokine production. *Vaccine*, 21, 19-20.
- Heale, V.R., Vanderwolf, C.H., Kavaliers, M. (1994). Components of weasel and fox odors elicit fast wave burst in the dentate gyrus of rats. *Behavioral Brain Research*, 63, 159-165.
- Heffelfinger, A.K., Newcomer, J.W. (2001). Glucocorticoid effects on memory function over the human life span. *Development and Psychopathology*, 13, 491-513.
- Helgeson, V.S. (1992). Moderators of the relationship between perceived control and adjustment to chronic illness. *Journal of Personality and Social Psychology*, 63, 656-666.
- Hemingway, H., Kuper, H., Marmot, M. (2003). Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1B (IL-1B), IL-6 and tumor necrosis factor-alpha (TNF $\alpha$ ) production in humans: high sensitivity of TNF $\alpha$  and resistance of IL-6. In Yusif, S., Cairns, J.A., Camm, A.J., Fallen, E.L., Gersh, B.J. (Eds.). *Evidence-Based Cardiology*. London; BMJ Books.
- Hemingway, H., Malik, M., Marmot, M. (2001). Social and psychological influences on sudden cardiac death, ventricular arrhythmia and cardiac autonomic function. *European Heart Journal*, 22, 1082-1101.
- Hess, T.M., Auman, C., Colcombe, S.J., Rahhal, T.A. (2003). The impact of stereotype threat on age differences in memory performance. *Journal of Gerontology: Psychological Sciences*, 58, 3-11.
- Hess, T.M., Rosenberg, D.C., Waters, S.J. (2001). Motivation and representational processes in adulthood: the effects of social accountability and information relevance. *Psychology and Aging*, 16, 629-642.
- Hett, S., Ramlow, G., Wolf, O.T. (2005). A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology*, 30, 771-784.

- Heyka, R.J. (1998). Obesity and hypertension. *Nutrition in Clinical Care*, 1, S30-S37.
- Higgins, M., Province, M., Heiss, G., Eckfeldt, J., Ellison, R.C., Folsom, A.R., Rao, D.C., Sprafka, J.M., Williams, R. (1996). NHLBI Family Health Study: objectives and design. *American Journal of Epidemiology*, 143, 1219-1228.
- Hingorani, A.D., Cross, J., Kharbanda, R.K., Mullen, M.J., Bhagat, K., Taylor, M., Donald, A.E., Palacios, M., Griffin, G.E., Deanfield, J.E., MacAllister, R.J., Vallance, P. (2000). Acute systemic inflammation impairs endothelium-dependent dilation in humans. *Circulation*, 102, 994-999.
- Hocking Schuler, J.L., O'Brien, W.H. (1997). Cardiovascular recovery from stress and hypertension risk factors: a meta-analytic review. *Psychophysiology*, 34, 649-659.
- Holmes, T.H., Rahe, R.H. (1967) The Social Readjustment Rating Scale. *Journal of Psychosomatic Research*, 11, 213-218.
- Horsten, M., Mittleman, M.A., Wamala, S.P., Schenck-Gustafsson, K., Orth-Gomer, K. (1999). Social relations and the metabolic syndrome in middle-aged Swedish women. *Journal of Cardiovascular risk*, 6, 391-397.
- Hubert, W., De Jong-Meyer, R. (1992). Salivary cortisol responses to unpleasant film stimuli differ between high and low trait anxious subjects. *Neuropsychology*, 25, 115-120.
- Humphries, S.E., Luong, L.A., Ogg, M.S., Hawe, E., Miller, G.J. (2001). The interleukin-6 174G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. *European Heart Journal*, 22, 2243-2252.
- Intengan, H.D., Schiffrin, E.L. (2001). Vascular remodelling in hypertension: roles of apoptosis, inflammation, and fibrosis. *Hypertension*, 38, 581-587.
- Introini-Collison, I.B., McGaugh, J.L. (1986). Epinephrine modulates long-term retention of an aversively motivated discrimination. *Behavioral & Neural Biology*, 45, 358-365.
- Irwin, M. (2002). Psychoneuroimmunology and depression: clinical implications. *Brain Behavior and Immunity*, 16, 1-16.
- Iverson, G.L. (2001). Interpreting change on the WAIS-III/WMS-III in clinical samples. *Archives of Clinical Neuropsychology*, 16, 183-191.
- Izquierdo-Porrera, A.M., Waldstein, S.R. (2002). Cardiovascular risk factors and cognitive function in African Americans. *Journal of Gerontology: Psychological Sciences*, 57B, 377-380.
- Jennings, J.R., Muldoon, M.F., Ryan, C.M., Mintum, M.A., Meltzer, C.C., Townsend, D.W., Sutton-Tyrell, K., Shapiro, A.P., Manuck, S.B. (1998). Cerebral blood flow in hypertensive patients: an initial report of reduced and compensatory blood flow responses during performance of two cognitive tasks. *Hypertension*, 31, 1216-1222.
- Jensen-Urstad, K., Storck, N., Bouvier, F., Ericson, M., Lindblad, L.E., Jensen-Urstad, M. (1997). Heart rate variability in healthy subjects is related to age and gender. *Acta Physiologica Scandinavica*, 160, 235-241.
- Jern, S., Bergbrant, A., Björntorp, P., Hansson, L. (1992). Relation of central hemodynamics to obesity and body fat distribution. *Hypertension*, 19, 520-527.

- Joëls, M. (1997). Steroids hormones and excitability in the mammalian brain. *Frontiers in Neuroendocrinology*, 18, 495-498.
- Joëls, M. (2000). Modulatory actions of steroid hormones and neuropeptides on electrical activity in brain. *European Journal of Pharmacology*, 405, 207-216.
- Joëls, M., De Kloet, E.R. (1994). Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. *Progress in Neurobiology*, 43, 1-36.
- Johansson, B. (1994). Pathogenesis of vascular dementia: the possible role of hypertension. *Dementia*, 5, 174-176.
- Johnson, E.H., Nazzaro, P., Gilbert, D.C. (1991). Cardiovascular reactivity to stress in black male offspring of hypertensive parents. *Psychosomatic Medicine*, 53, 420-432.
- Joint Health Survey Unit. (2005). *Health Survey for England 2003*. London; The Stationary Office.
- Jorgenson, R.S., Gelling, P.D., Kliner, L. (1992). Patterns of social desirability and anger in young men with a parental history of hypertension: association with cardiovascular activity. *Health Psychology*, 11, 403-412.
- Kalmijn, S., Launer, L.J., Stolk, R.P., De Jong, F.H., Pols, H.A., Hofman, A., Breteler, M.M., Lamberts, S.W. (1998). A prospective study on cortisol, dehydroepiandrosterone sulfate, and cognitive function in the elderly. *Journal of Clinical Endocrinology and Metabolism*, 83, 3487-3492.
- Kamarck, T.W., Lovallo, W.R. (2003). Cardiovascular reactivity to psychological challenge: conceptual and measurement considerations. *Psychosomatic Medicine*, 65, 9-21.
- Kang, D.-H., Fox, C. (2001). 38Th1 and Th2 cytokine responses to academic stress. *Research in Nursing and Health*, 24, 245-257.
- Kapadia, S.R. (1999). Cytokines and heart failure. *Cardiovascular Review*, 7, 196-206.
- Karlamangla, A.S., Singer, B., McEwen, B., Rowe, J.W., Seeman, T.E. (2002). Allostatic load as a predictor of functional decline: MacArthur studies of successful aging. *Journal of Clinical Epidemiology*, 55, 696-710.
- Kasagi, F., Akahoshi, M., Shimaoka, K. (1995). Relation between cold pressor test and development of hypertension based on 28-year follow-up. *Hypertension*, 25, 71-76.
- Katsuura, G., Arimura, A., Koves, K., Gottschall, P.E. (1990). Involvement of organum vasculosum of lamina terminalis and preoptic area in interleukin 1 beta-induced ACTH release. *American Journal of Physiology*, 258, 163-171.
- Katz, M.H. (1999). *Multivariate Analysis: a Practical Guide for Clinicians*. Cambridge; Cambridge University Press.
- Kaufman, A.S., Lichenberger, E.O. (1999). *Essentials of the WAIS-III Assessment*. New York; John Wiley & Sons, Inc.
- Kelley, D.E. (1998). Managing obesity as first-line therapy for diabetes mellitus. *Nutrition in Clinical Care*, 1, S38-S43.

- Kelley, K.W., Bluthé, R.-M., Dantzeré, R., Zhou, J.-H., Shen, W.-H., Johnson, R.W., Broussard, S.R. (2003). Cytokine induced sickness behavior. *Brain Behavior and Immunity*, 17, S112-S118.
- Kelly, K.S., Hayslip, B., Servaty, H.L. (1996). Psychoneuroendocrinological indicators of stress and intellectual performance among older adults. *Experimental Aging Research*, 22, 393-401.
- Kerr, D.S., Campbell, L.W., Hao, S.-Y., Landfield, P.W. (1989). Corticosteroid modulation of hippocampal potentials: Increased effect with aging. *Science*, 245, 1505-1509.
- Ketterer, M.W., Freedland, K.E., Krantz, D.S., Kaufman, P., Forman, S., Greene, A., Raczyński, J., Knatterud, G., Light, K., Carney, R.M., Stone, P., Becker, L., Sheps, D. (2000). Psychological correlates of mental stress-induced ischemia in the laboratory: the psychological investigation of myocardial ischemia (PIMI) study. *Journal of Health Psychology*, 5, 75-85.
- Kharbanda, R.K., Walton, B., Allen, M., Klein, N., Hingorani, A.D., MacAllister, R.J., Vallance, P. (2002). Prevention and inflammation-induced endothelial dysfunction: a novel vasculo-protective action of aspirin. *Circulation*, 105, 2600-2604.
- Kiecolt-Glaser, J.K., Preacher, K.J., MacCallum, R.C., Atkinson, C., Malarkey, W., Glaser, R. (2003). Chronic stress and age related increases in proinflammatory cytokine IL-6. *Proceedings of the national academy of sciences*, 100, 9090-9095.
- Kirschbaum, C., Hellhammer, D.H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*, 19, 313-333.
- Kirschbaum, C., Prussner, J.C., Stone, A.A., Federnko, I., Gaab, D., Lintz, N., Schommer, N., Hellhammer, D.H. (1995). Persistent high cortisol response to repeated psychological stress in a subpopulation of healthy men. *Psychosomatic Medicine*, 57, 468-474.
- Kirschbaum, C., Wolf, O.T., May, M., Wippich, W., Hellhammer, D.H. (1996). Stress and treatment induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sciences*, 58, 1475-1483.
- Kluger, M.J. (1991). Fever: role of pyrogens and cryogens. *Physiological Reviews*, 71, 93-127.
- Kneipp, S.M., Drevdahl, D.J. (2003). Problems with parsimony in research on socioeconomic determinants of health. *Advances in Nursing Science*, 26, 162-172.
- Konsman, J.P., Parnet, P., Dantzer, R. (2002). Cytokine-induced sickness behavior: mechanisms and implications. *Trends in Neuroscience*, 25, 154-159.
- Kop, W.J., Gottdiener, J.S. (2005). The role of immune system parameters in the relationship between depression and coronary artery disease. *Psychosomatic Medicine*, 67, S37-S41.
- Kop, W.J., Gottdiener, J.S., Tangen, C.M., Fried, L.P., McBurnie, M.A., Walston, J., Newman, A., Hirsch, C., Tracy, R.P. (2002). Inflammation and coagulation factors in persons >65 years of age with symptoms of depression but without evidence of myocardial ischemia. *The American Journal of Cardiology*, 89, 419-424.
- Kreipe, R.E., Goldstein, B., De King, D.E., Tipton, R., Kempinski, M.H. (1994). Heart rate power spectrum and autonomic dysfunction in adolescents with anorexia nervosa. *International Journal of Eating Disorders*, 16, 159-165.

- Kubzansky, L.D., Berkman, L.F., Glass, T.A., Seeman, T.E. (1998). Is educational attainment associated with shared determinants of health in the elderly? findings from the MacArthur studies of successful aging. *Psychosomatic Medicine*, 60, 578-585.
- Kudielka, B.M., Broderick, J.E., Kirschbaum, C. (2003). Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic Medicine*, 65, 313-319.
- Kudielka, B.M., Hellhammer, J., Hellhammer, D.H., Wolf, O.T., Pirke, K.M., Varadi, E., Pulz, J., Kirschbaum, C. (1998). Sex differences in endocrine and psychological responses to psychosocial stress in healthy elderly subjects and the impact of a 2-week DHEA treatment. *Journal of Clinical Endocrinology and Metabolism*, 83, 1756-1761.
- Kudielka, B.M., Kirschbaum, C. (2003). Awakening Cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology*, 28, 35-47.
- Kudielka, B.M., Schmidt-Reinwald, A.K., Hellhammer, D.H., Kirschbaum, C. (1999). Psychological and endocrine responses to psychosocial stress and Dex-CRF in healthy postmenopausal women and young controls: the impact of age and a two-week estradiol treatment. *Neuroendocrinology*, 70, 422-430.
- Kudielka, B.M., Schmidt-Reinwald, A.K., Hellhammer, D.H., Kirschbaum, C. (2000). Psychosocial stress and functioning of the hypothalamic-pituitary-adrenal axis: no evidence for a reduced resilience in elderly men. *Stress*, 3, 229-240.
- Kunz-Ebrecht, S.R., Kirschbaum, C., Marmot, M., Steptoe, A. (2004a). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology*, 29, 516-528.
- Kunz-Ebrecht, S.R., Kirschbaum, C., Steptoe, A. (2004b). Work stress, socioeconomic status and neuroendocrine activation over the working day. *Social Science and Medicine*, 58, 1523-1530.
- Kupper, N., De Geus, E.J.C., Van Den Berg, M., Kirschbaum, C., Boomsma, D.I., Willemsen, G. (2005). Familial influences on basal salivary cortisol in an adult population. *Psychoneuroendocrinology*, 30, 857-868.
- Lachman, M.E., Weaver, S.L. (1998). The sense of control as a moderator of social class differences in health and well-being. *Journal of Personality and Social Psychology*, 74, 763-773.
- Lamensdorf, A.M., Linden, W. (1992). Family history of hypertension and cardiovascular changes during high and low affect provocation. *Psychophysiology*, 29, 558-565.
- Lamine, S.B., Calabrese, P., Perrault, H., Dinh, T.P., Eberhard, A., Benchetrit, G. (2004). Individual differences in respiratory sinus arrhythmia. *American Journal of Physiology: Heart and Circulation Physiology*, 286, 2305-2313.
- Landfield, P., Baskin, R.W., Pitler, T.A. (1981). Brain-aging correlates: retardation by hormonal-pharmacological treatments. *Science*, 214, 581-584.
- Landfield, P., Waymire, J., Lynch, G. (1978). Hippocampal aging and adrenocorticoids: a quantitative correlation. *Science*, 202, 1098-1102.

- Lantz, P.M., Lynch, J.W., House, J.S., Lepkowski, J.M., Mero, R.P., Musick, M.A., Williams, D.R. (2001). Socioeconomic disparities in health change in a longitudinal study of US adults: the role of health-risk behaviors. *Social Science & Medicine*, 53, 29-40.
- Larson, S.J., Dunn, A.J. (2001). Behavioral effects of cytokines. *Brain Behavior and Immunity*, 15, 371-387.
- Lathe, R. (2001). Hormones and the hippocampus. *Journal of Endocrinology*, 169, 205-231.
- Launer, L.J., Masaki, K., Petrovitch, H., Foley, D., Havlik, R.J. (1995). The association between midlife blood pressure levels and late-life cognitive function: the Honolulu-Asia Aging Study. *Journal of the American Medical Association*, 274, 1846-1851.
- Lawler, K.A., Kline, K., Seabrook, E., Krishnamoorthy, J., Anderson, S.F., Wilcox, Z.C., Craid, F., Adlin, R., Thomas, S. (1998). Family history of hypertension: a psychophysiological analysis. *International Journal of Psychophysiology*, 28, 207-222.
- Lazarus, R.S., Folkman, S. (1984). *Stress, Coping and Appraisal*. New York; Springer-Verlag.
- Leal-Cerro, A., Soto, A., Martinez, M.A., Dieguez, C., Casanueva, F.F. (2001). Influence of cortisol status on leptin secretion. *Pituitary*, 4, 111-116.
- Lee, D.L., Leite, R., Fleming, C., Pollock, J.S., Webb, R.C., Brands, M.W. (2004). Hypertensive response to acute stress is attenuated in interleukin-6 knockout mice. *Hypertension*, 44, 259-263.
- Lehrer, P.M., Hochron, S., Carr, R., Edelberg, R., Hamer, r., Jackson, A., Porges, S. (1996). Behavioral task-induced bronchodilation in asthma during active and passive tasks: a possible cholinergic link to psychologically-induced airway changes. *Psychosomatic Medicine*, 58, 413-422.
- Lesch, K.P., Bengel, D., Heiels, A., Sabol, S.Z., Greenberg, B., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274, 1527-1531.
- Lett, H.S., Blumenthal, J.A., Babyak, M.A., Sherwood, A., Stauman, T., Robins, C., Newman, M.F. (2004). Depression as a risk factor for coronary artery disease: evidence, mechanisms, and treatment. *Psychosomatic Medicine*, 66, 305-315.
- Levy, B. (1996). Improving memory in old age by implicit self-stereotyping. *Journal of Personality and Social Psychology*, 71, 1092-1107.
- Levy, B., Hausdorff, J.M., Hencke, R., Wei, J.Y. (2000). Reducing cardiovascular stress with positive self-stereotypes of aging. *Journal of Gerontology: Psychological Sciences*, 55, 205-213.
- Li, G.-H., Faulhaber, H.-D., Rosenthal, M., Schuster, H., Jordan, J., Timmerman, B., Hoehe, M.R., Luft, F.C., Busjahn, A. (2001).  $\beta$ -2 adrenergic receptor gene variations and blood pressure under stress in normal twins. *Psychophysiology*, 38, 485-489.
- Liang, K.C., Juler, R.G., McGaugh, J.L. (1986). Modulating effects of post-training epinephrine on memory: Involvement of the amygdala noradrenergic system. *Brain Research*, 368, 125-133.
- Libby, P., Ridker, P.M., Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation*, 105, 1135-1143.

- Licinio, J., Mantzoros, C., Negrao, A.B., Cizza, G., Wong, M.L., Bongiorno, P.B., Chrousos, G.P., Karp, B., Allen, C., Flier, J.S., Gold, P.W. (1997). Human Leptin concentrations are pulsatile and inversely related to pituitary-adrenal function. *Nature Medicine*, 3, 575-579.
- Light, K.C., Dolan, C.A., Davis, M.R., Sherwood, A. (1992). Cardiovascular response to an active coping challenge as predictors of blood pressure patterns 10 to 15 years later. *Psychosomatic Medicine*, 54, 217-230.
- Linden, W., Earle, T.L., Gerin, W., Christenfeld, N. (1997). Physiological stress reactivity and recovery: conceptual siblings separated at birth? *Journal of Psychosomatic Research*, 42, 117-135.
- Linden, W., Gerin, W., Davidson, K.W. (2003). Cardiovascular reactivity: status quo and a research agenda for the new millennium. *Psychosomatic Medicine*, 65, 5-8.
- Lindmark, E., Diderholm, E., Wallentin, L., Siegbahn, A. (2001). Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive and non-invasive strategy. *Journal of the American Medical Association*, 286, 2107-2113.
- Ling, M., Perry, P., Tsuang, M. (1981). Side effects of corticosteroid therapy. *Archives of General Surgery*, 38, 471-477.
- Lischner, M., Akselrod, S., Mor Avi, V., Oz, O. (1987). Spectral analysis of heart rate fluctuations. A non-invasive, sensitive method for the early diagnosis of autonomic neuropathy in diabetes mellitus. *Journal of the Autonomic Nervous System*, 19, 119-125.
- Litchenberger, E.O., Kaufman, A.S., Lai, Z.C. (2002). *Essentials of WMS-III Assessment*. New York; John Wiley & Sons, Inc.
- Ljung, T., Holm, G., Friberg, P., Andersson, B., Bengtsson, B.-A., Svensson, J., Dallman, M.F., McEwen, B., Björntorp, P. (2000). The activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in relation to waist/hip circumference ratio in men. *Obesity Research*, 8, 487-495.
- Lorant, V., Delige, D., Eaton, W., Robert, A., Philippot, P., Ansseau, M. (2003). Socioeconomic inequalities in depression: a meta-analysis. *American Journal of Epidemiology*, 157, 98-112.
- Lovallo, W.R. (2004). *Stress and Health : Biological and Psychological Interactions*. London; Sage.
- Lovallo, W.R., Gerin, W. (2003). Psychophysiological reactivity: mechanisms and pathways to cardiovascular. *Psychosomatic Medicine*, 65, 36-45.
- Lovallo, W.R., Wilson, M.F. (1992a). A biobehavioral model of hypertension development. In Turner, J.R., Sherwood, A., Light, K.C. (Eds.). *Individual Differences in Cardiovascular Response to Stress: Perspectives on Individual Differences*. New York; Plenum Press.
- Lovallo, W.R., Wilson, M.F. (1992b). The role of cardiovascular reactivity in hypertension risk. In Turner, J.R., Sherwood, A., Light, K.C. (Eds.). *Individual Differences in Cardiovascular Response to Stress: Perspectives on Individual Differences*. New York; Plenum Press.



- Lupien, S.J., De Leon, M., De Santi, S., Convit, A., Tarshish, C., Nair, N.P.V., McEwen, B.S., Hauger, R.L., Meaney, M.J. (1998). Longitudinal increase in cortisol during human aging predicts hippocampal atrophy and memory deficits. *Nature Neuroscience*, 1, 69-73.
- Lupien, S.J., Fiocco, A., Wan, A., Maheu, F., Lord, C., Schramek, T., Tu, M.T. (2005). Stress hormones and human memory function across the lifespan. *Psychoneuroendocrinology*, 30, 225-242.
- Lupien, S.J., Gaudreau, S., Tchiteya, B.M., Maheu, F., Sharma, S., Nair, N.P.V., Hauger, R.L., McEwen, B.S., Meaney, M.J. (1997). Stress-induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity. *Journal of Clinical Endocrinology and Metabolism*, 82, 2070-2075.
- Lupien, S.J., King, S., Meaney, M.J., McEwen, B. (2000). Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biological Psychiatry*, 48, 976-980.
- Lupien, S.J., King, S., Meaney, M.J., McEwen, B. (2001). Can poverty get under your skin? Basal cortisol levels and cognitive function in children from low and high socioeconomic status. *Development & Psychopathology*, 13, 653-676.
- Lupien, S.J., Lecours, A.R., Lussier, I., Schwartz, G., Nair, N.P.V., Meaney, M.J. (1994). Basal cortisol levels and cognitive deficits in aging humans. *Journal of Neuroscience*, 14, 2893-2903.
- Lupien, S.J., Lecours, A.R., Schwartz, G., Sharma, S., Hauger, R.L., Meaney, M.J., Nair, N.P.V. (1995). Longitudinal study of basal cortisol levels in healthy elderly subjects: evidence for subgroups. *Neurobiology of Aging*, 17, 95-105.
- Lupien, S.J., Lepage, M. (2001). Stress, memory, and the hippocampus: can't live with it, can't live without it. *Behavioral Brain Research*, 127, 137-158.
- Lupien, S.J., McEwen, B.S. (1997). The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Research Reviews*, 24, 1-27.
- Lupien, S.J., Wilkinson, C.W., Brière, S., Ng Ying Kin, N.M.K., Meaney, M.J., Nair, N.P.V. (2002). Acute modulation of aged human memory by pharmacological manipulation of glucocorticoids. *Journal of Clinical Endocrinology and Metabolism*, 87, 3798-3807.
- Lutgendorf, S.K., Garand, L., Buckwalter, K.C., Reimer, T.T., Hong, S.-Y., Lubaroff, D.M. (1999). Life stress, mood disturbance, and elevated interleukin-6 in healthy older women. *Journals of Gerontology Series A - Biological Sciences & Medical Sciences*, 54, 343-349.
- Lynch, J.W., Kaplan, G.A., Shema, S.J. (1997). Cumulative impact of sustained economic hardship on physical, cognitive, psychological and social functioning. *New England Journal of Medicine*, 337, 1889-1895.
- Lyonfield, J.D., Borkovec, T.D., Thayer, J.F. (1995). Vagal tone in generalized anxiety disorder and effects of aversive imagery and worrisome thinking. *Behavior Therapy*, 26, 457-466.
- MacLulich, A.M.J., Deary, I.J., Starr, J.M., Ferguson, K.J., Wardlaw, J.M., Seckl, J.R. (2005). Plasma cortisol levels, brain volumes and cognition in healthy elderly men. *Psychoneuroendocrinology*, 30, 505-515.

- MacMahon, S. (1987). Alcohol consumption and hypertension. *Hypertension*, 9, 111-121.
- Maes, M., Bosmans, E., Meltzer, H.Y. (1993a). Interleukin-1 beta: a putative mediator of HPA axis hyperactivity in major depression? *American Journal of Psychiatry*, 150, 1189-1193.
- Maes, M., Lin, A.-H., Delmeire, L., Van Gastel, A., Kenis, G., De Jongh, R., Bosmans, E. (1999). Elevated serum interleukin-6 (IL-6) and IL-6 receptor concentrations in posttraumatic stress disorder following accidental man-made traumatic events. *Biological Psychiatry*, 45, 833-839.
- Maes, M., Scharpé, S., Meltzer, H.Y., Bosmans, E., Suy, E., Calabrese, J., Cosyns, P. (1993b). Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pituitary-adrenal axis in severe depression. *Psychiatry Research*, 49, 11-27.
- Maes, M., Song, C., Lin, A., De Jongh, R., Van Gastel, A., Kenis, G., Bosmans, E., De Meester, I., Benoy, I., Neels, H., Demedts, P., Janca, A., Scharpé, S., Smith, R.S. (1998). The effects of psychological stress on humans: increased production of proinflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine*, 10, 313-318.
- Maes, M., Vandoolaeghe, E., Ranjan, R., Bosmans, E., Bergmans, R., Desnyder, R. (1995). Increased serum interleukin-1-receptor-antagonist concentrations in major depression. *Journal of Affective Disorders*, 36, 29-36.
- Maheu, F., Joobert, R., Beaulieu, S., Lupien, S.J. (2004). Differential effects of adrenergic and corticosteroid hormonal systems on human short and long term declarative memory for emotionally arousing material. *Behavioral Neuroscience*, 118, 420-428.
- Maier, S.F., Watkins, L.R. (1998). Cytokines for psychologists: Implications of bidirectional immune-to-brain communication for understanding behavior, mood and cognition. *Psychological Review*, 105, 83-107.
- Maier, S.F., Watkins, L.R. (1999). Bidirectional communication between the brain and the immune system: implications for behaviour. *Animal Behaviour*, 57, 741-751.
- Makris, T.K., Tsoukala, C., Krespi, P., Hatzizacharias, A., Gialeraki, A., Papargyriou, J., Votteas, V., Mandalaki, T. (1997). Haemostasis balance disorders in patients with essential hypertension. *Thrombosis Research*, 88, 99-107.
- Manger, K., Kusus, M., Forster, C., Ropers, D., Daniel, W.G., Kalden, J.R., Achenbach, S., Manger, B. (2003). Factors associated with coronary artery calcification in young female patients with SLE. *Annals of the Rheumatic Diseases*, 62, 846-850.
- Manuck, S.B. (1994). Cardiovascular reactivity in cardiovascular disease: 'once more into the breach'. *International Journal of Behavioral Medicine*, 1, 4-31.
- Manuck, S.B., Kamarck, T.W., Kasprowicz, A.S., Waldstein, S.R. (1993). Stability and patterning of behaviorally evoked cardiovascular reactivity. In Blascovich, J., Katkin, S.E. (Eds.). *Cardiovascular Reactivity to Psychological Stress and Disease*. Washington DC; American Psychological Association.
- Manuck, S.B., Kaplan, J.R., Muldoon, M.F., Adams, M.R., Clarkson, T.B. (1991). The behavioral exacerbation of atherosclerosis and its inhibition by propranolol. In McCabe, P.M., Schneidermann, N., Field, T.M., Skyler, J.S. (Eds.). *Stress, Coping and Disease*. New Jersey; Erlbaum.

- Manuck, S.B., Kasprowicz, A.S., Muldoon, M.F. (1990). Behaviorally-evoked cardiovascular reactivity and hypertension: conceptual issues and potential associations. *Annals of Behavioral Medicine*, 12, 17-29.
- Manuck, S.B., Marsland, A.L., Kaplan, J.R., Williams, J.K. (1995). The pathogenicity of behavior and its neuroendocrine mediation: An example from coronary artery disease. *Psychosomatic Medicine*, 57, 275-283.
- Manuck, S.B., Polefrone, J.M., Terrell, D.F., Muldoon, M.F., Kasprowicz, A.L., Waldstein, S.R., Jennings, J.R., Malkoff, S.B., Marsland, A., Graham, R.E. (1996). Absence of enhanced sympathoadrenal activity and behaviorally evoked cardiovascular reactivity among offspring of hypertensives. *American Journal of Hypertension*, 9, 245-255.
- Marin, P., Darin, N., Amemiya, T., Andersson, B., Jern, S., Björntorp, P. (1992). Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism*, 41, 882-886.
- Markovitz, J.H., Raczynski, J., Wallace, D., Chettur, V., Chesney, M.A. (1998). Cardiovascular reactivity to video game predicts subsequent blood pressure increases in young men: the CARDIA Study. *Psychosomatic Medicine*, 60, 186-191.
- Marmot, M.G., Davey Smith, G., Stansfeld, S., Patel, C., North, F., Head, J., White, I., Brunner, E., Feeney, A. (1991). Health inequalities among British civil servants: the Whitehall II study. *Lancet*, 337, 1387-1393.
- Marmot, M.G., Shipley, M.J. (1996). Do socioeconomic differences in mortality persist after retirement? 25 year follow up of civil servants from the first Whitehall study. *British Medical Journal*, 313, 1177-1180.
- Marrero, A.F., Al' Absi, M., Pincomb, G.A., Lovallo, W.R. (1997). Men at risk for hypertension show elevated vascular resistance at rest and during mental stress. *International Journal of Psychophysiology*, 25, 185-192.
- Martignoi, E., Petrglia, F., Costa, A., Bono, G., Genazzani, A.R., Nappi, G. (1990). Dementia of the Alzheimer's type and the hippocampal-pituitary-adrenocortical axis: changes in cerebrospinal fluid, corticotrophin releasing factor and plasma cortisol levels. *Acta Neurologica Scandinavica*, 81, 452-456.
- Matthews, K.A., Rakaczky, C.J. (1986). Familial aspects of the Type A behavior pattern and physiological reaction to stress. In Schmidt, T.H., Dembroski, T.M. (Eds.). *Factors in Cardiovascular Disease*. Berlin; Springer.
- Matthews, K.A., Woodall, K.L., Allen, M.T. (1993). Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension*, 22, 479-485.
- Mauri, M., Sinforiani, E., Bono, G., Vignati, F., Berselli, M.E., Attanasio, R., Nappi, G. (1993). Memory impairment in Cushing's disease. *Acta Neurologica Scandinavica*, 87, 52-55.
- Maver, J., Strucl, M., Accetto, R. (2004). Autonomic nervous system activity in normotensive subjects with a family history of hypertension. *Clinical Autonomic Research*, 14, 369-375.
- McAdoo, W.G., Weinberger, M.H., Miller, J.Z., Fineberg, N.S., Grim, C.E. (1990). Race and gender influence hemodynamic responses to psychological and physical stimuli. *Journal of Hypertension*, 8, 961-967.

- McCaffery, J.M., Bleil, M., Pogue-Geile, M.F., Ferrell, R.E., Manuck, S.B. (2003). Allelic variation in the serotonic transporter gene-linked polymorphic region (5-HTTLPR) and cardiovascular reactivity in young adult male and female twins of European-American descent. *Psychosomatic Medicine*, 65, 721-728.
- McCaffery, J.M., Pogue-Geile, M.F., Ferrell, R.E., Petro, N., Manuck, S.B. (2002). Variability within  $\alpha$ - and  $\beta$ -adrenoreceptor genes as a predictor of cardiovascular function at rest and in response to mental challenge. *Journal of Hypertension*, 20, 1105-1106.
- McCann, S.J., Sterwin, L.L., Short, R.H. (1991). Sex differences, social desirability, masculinity and the tendency to worry. *Journal of Geriatric Psychology*, 152, 295-301.
- McEwen, B. (1998). Stress, adaptation and disease. *Annals of the New York Academy of Sciences*, 840, 33-44.
- McEwen, B. (2000). Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology*, 22, 108-124.
- McEwen, B., De Leon, M., Lupien, S.J., Meaney, M.J. (1999). Corticosteroids, the aging brain and cognition. *Trends in Endocrinology and metabolism*, 10, 92-96.
- McEwen, B., Wingfield, J.C. (2003a). Response to commentaries on concept of allostasis. *Hormones and Behavior*, 43, 28-30.
- McEwen, B., Wingfield, J.C. (2003b). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, 43, 2-15.
- McEwen, B.S. (2002). Sex, stress and the hippocampus: allostasis, allostatic load and the ageing process. *Neurobiology of Aging*, 23, 921-939.
- McEwen, B.S. (2003). Interacting mediators of allostasis and allostatic load: towards an understanding of resilience in aging. *Metabolism*, 52, 10-16.
- McEwen, B.S., Alves, S.E., Bulloch, K., Weiland, N.G. (1997a). Ovarian steroids and the brain: implications for cognition and aging. *Neurology*, 48, S8-S15.
- McEwen, B.S., Biron, C.A., Brunson, K.W., Bulloch, K., Chambers, W.H., Dhabhar, F.S., Goldfarb, R.H., Kitson, R.P., Miller, A.H., Spencer, R.L., Weiss, J.M. (1997b). The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Research - Brain Research Reviews*, 23, 79-133.
- McEwen, B.S., Sapolsky, R.M. (1995). Stress and cognitive function. *Current Opinion in Neurobiology*, 5, 205-216.
- McEwen, B.S., Seeman, T.E. (1999). Protective and damaging effect of mediators of stress: elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences*, 869, 30-47.
- McEwen, B.S., Stellar, E. (1993). Stress and the individual: mechanisms leading to disease. *Archives of Internal Medicine*, 153, 2093-2101.
- McGeer, P.L., McGeer, P.L. (1995). The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative disorders. *Brain Research Reviews*, 21, 195-218.

- McGinnis, J.M., Foege, W.H. (1993). Actual causes of death in the United States. *Journal of the American Medical Association*, 270, 2207-2212.
- McNair, D.M., Lorr, N., Droppleman, L.F. (1981). Manual for the Profile of Mood States. San Diego, CA; Education and Industrial Testing Service.
- McNeilly, M., Anderson, N.B. (1996). Age differences in physiological response to stress. In Ruskin, P.E., Talbott, J.A. (Eds.). *Aging and Posttraumatic Stress Disorder*. London; American Psychiatric Press.
- Meaney, M.J., Mitchell, J.B., Aitken, D.H., Bhatnagar, S., Bodnoff, S.R., Iny, L.J., Sarrieau, A. (1991). The effects of neonatal handling on the development of the adrenocortical response to stress: implications from neuropathology and cognitive deficits in later life. *Psychoneuroendocrinology*, 16, 85-103.
- Meaney, M.J., O'Donnell, D., Rowe, W., Tannenbaum, B., Steverman, A., Walker, M., Nair, N.P.V., Lupien, S.J. (1995). Individual differences in hypothalamic-pituitary-adrenal activity in later life and hippocampal aging. *Experimental Gerontology*, 30, 229-251.
- Melanson, K.J., McInnis, K.J., Rippe, J.M., Blackburn, G., Wilson, P.F. (2001). Obesity and cardiovascular disease risk: research update. *Cardiology in Review*, 9, 202-207.
- Melzer, D., McWilliams, B., Brayne, C., Johnson, T., Bond, T. (2000). Socioeconomic status and the expectation of disability in old age: estimates for England. *Journal of Epidemiology and Community Health*, 54, 286-292.
- Menkes, M.S., Matthews, K.A., Krantz, D.S., Lundberg, V., Mead, L.A., Qaqish, B., Liang, K.Y., Thomas, C.B., Pearson, T.A. (1989). Cardiovascular reactivity to the cold pressor test as a predictor of hypertension. *Hypertension*, 14, 524-430.
- Michelson, D., Stratakis, C., Hill, L., Reynolds, J., Galliven, E., Chrousos, G., Gold, P. (1996). Bone mineral density in women with depression. *New England Journal of Medicine*, 335, 1176-1181.
- Mickelson, K.D., Kubansky, L.D. (2003). Social distribution of social support: the mediating role of life events. *American Journal of Community Psychology*, 32, 268-281.
- Miller, A.H., Sastry, G., Speranza, A.J.Jr., Lawlor, B.A., Mohs, R.C., Ryan, T.M., Gabel, S.M., Serby, M., Schmeidler, J., Davis, K.L. (1994). Lack of association between cortisol hypersecretion and nonsuppression on the DST in patients with Alzheimer's disease. *American Journal of Psychiatry*, 151, 267-270.
- Miller, G.E., Freedland, K.E., Carney, R.M., Stetler, C.A., Banks, W.A. (2003). Pathways linking depression, adiposity, and inflammation markers in healthy young adults. *Brain Behavior and Immunity*, 17, 276-285.
- Miller, S.B. (1992). Affective moderators of the cardiovascular response to stress in offspring of hypertensives. *Journal of Psychosomatic Research*, 36, 149-157.
- Miller, S.B. (1994). Parasympathetic nervous system control of heart rate responses to stress in offspring of hypertensives. *Psychophysiology*, 31, 11-16.
- Miller, S.B., Ditto, B. (1991). Exaggerated sympathetic nervous system response to extended psychological stress in offspring of hypertensives. *Psychophysiology*, 28, 103-113.
- Miller, S.B., Friese, M., Sita, A. (1995). Parental history of hypertension, sodium loading, and cardiovascular response to stress. *Psychosomatic Medicine*, 57, 381-389.

- Miller, S.B., Sita, A. (1994). Parent history of hypertension, menstrual cycle phase, and cardiovascular response to stress. *Psychosomatic Medicine*, 56, 61-69.
- Mills, P.J., Berry, C.C., Dimsdale, J.E., Nelesen, R.A., Ziegler, M.G. (1993). Temporal stability of task-induced cardiovascular adrenergic, and psychological responses: the effects of race and hypertension. *Psychophysiology*, 30, 197-204.
- Mills, P.J., Berry, C.C., Dimsdale, J.E., Ziegler, M.G., Nelesen, R.A., Kennedy, B.P. (1995). Lymphocyte subset redistribution in response to acute experimental stress: effects of gender, ethnicity, hypertension, and sympathetic nervous system. *Brain Behavior and Immunity*, 9, 61-69.
- Minuz, P., Patrignani, P., Gaino, S., Seta, F., Capone, M.L., Tacconelli, S., Degan, M., Faccini, G., Fornasiero, A., Talamini, G., Tonmasoli, R., Arosio, E., Santonastaso, C.L., Lechi, A., Patrono, C. (2004). Determinants of platelet activation in human essential hypertension. *Hypertension*, 43, 64-70.
- Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D.R., Miles, J.M., Yudkin, J.S., Klein, S., Coppack, S.W. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *Journal of Clinical Endocrinology and Metabolism*, 82, 4196-4200.
- Muldoon, M.F., Terrell, D.F., Bunker, C.H., Manuck, S.B. (1993). Family history of studies in hypertension research: review of the literature. *American Journal of Hypertension*, 6, 76-88.
- Muldoon, M.F., Waldstein, S.R., Ryan, C.M., Jennings, J.R., Polefrone, J.M., Shapiro, A.P., Manuck, S.B. (2002). Effects of six anti-hypertensive medications on cognitive performance. *Journal of Hypertension*, 20, 1643-1652.
- Muller, J.E., Tofler, G.H. (1990). A symposium: triggering and circadian variation of onset of acute cardiovascular disease. *American Journal of Cardiology*, 66, 11-70.
- Muna, W.F.T. (1993). Cardiovascular disorders in Africa. *World Statistics Quarterly*, 46, 125-133.
- Murphy, J.K., Alpert, B.S., Walker, S.S. (1992). Ethnicity, pressor reactivity, and children's blood pressure. *Hypertension*, 20, 327-332.
- Musselman, D.L., Miller, A.H., Porter, M.R., Manatunga, A., Gao, F., Penna, S., Pearce, B., Pearce, B.D., Landry, J., Glover, S., McDaniel, J.S., Nemeroff, C.B. (2001). Higher than normal plasma Interleukin-6 concentrations in cancer patients with depression: preliminary findings. *American Journal of Psychiatry*, 158, 1252-1257.
- Myint, A.M., Kim, Y.K. (2003). Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. *Medical Hypotheses*, 61, 519-525.
- Nagai, N., Matsumoto, T., Kita, H., Moritani, T. (2003). Autonomic nervous system activity and the state and development of obesity in Japanese school children. *Obesity Research*, 11, 25-32.
- National Audit Office. (2001). Tackling Obesity in England. *House of Commons 220, Session 2000-2001*, 1-63.
- Nelesen, R.A., Shaw, R., Ziegler, M.G., Dimsdale, J.E. (1999). Impedance cardiography-derived hemodynamic responses during baroreceptor testing with amyl nitrite and phenylephrine: A validity and reliability study. *Psychophysiology*, 36, 105-108.

- Newcomer, J.W., Selke, G., Melson, A.K., Hershey, T., Craft, S., Richards, K., Alderson, A.L. (1999). Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Archives of General Psychiatry*, 56, 527-533.
- Newman, J.D., McGarvey, S.T., Steele, M.S. (1999). Longitudinal association of cardiovascular reactivity and blood pressure in Samoan adolescents. *Psychosomatic Medicine*, 61, 243-249.
- Nicolson, N., Storms, C., Ponds, R., Sulon, J. (1997). Salivary cortisol levels and stress reactivity in human aging. *Journal of Gerontology*, 52, 68-75.
- Nye, E.J., Borstein, S.R., Grice, J.E., Tauchnitz, R., Hockings, G.I., Strakosch, C.R., Jackson, R.V., Torpy, D.J. (2000). Interactions between the stimulated hypothalamic-pituitary-adrenal axis and leptin in humans. *Journal of Neuroendocrinology*, 12, 141-145.
- O'Brien, J., Desmond, P., Ames, D., Schweitzer, I., Harrigan, S., Tress, B. (1996). A magnetic resonance imaging study of white matter lesions in depression and Alzheimer's disease. *British Journal of Psychiatry*, 168, 477-485.
- O'Brien, W.H., Haynes, S.N., Mumby, P.B. (1998). Differences in cardiovascular recovery among healthy young adults with and without a parental history of hypertension. *Journal of Psychophysiology*, 12, 17-28.
- Odio, M., Brodish, A. (1989). Age-related adaptation of pituitary-adrenocortical responses to stress. *Neuroendocrinology*, 49, 382-388.
- Office for National Statistics. (2005). *2003/2004 General Household Survey*. [www.ons.gov.uk/ghs](http://www.ons.gov.uk/ghs).
- Operario, D., Adler, N.E., Williams, D.R. (2004). Subjective social status: Reliability and predictive utility for global health. *Psychology and Health*, 19, 237-246.
- Orshal, J.M., Khalil, R.A. (2004). Reduced endothelial NO-cGMP mediated vascular relaxation and hypertension in IL-6 infused pregnant rats. *Hypertension*, 43, 434-444.
- Otte, C., Hart, S., Neylan, T.C., Marmar, C.R., Yaffe, K., Mohr, D.C. (2005). A meta-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology*, 30, 80-91.
- Owen, N., Steptoe, A. (2003). Natural killer cell and proinflammatory cytokine responses to mental stress: associations with heart rate variability. *Biological Psychology*, 63, 101-115.
- Oxenkrug, G.F., Gurevich, D., Siegel, B., Dumiao, M.S., Gershon, S. (1999). Correlation between brain-adrenal axis activation and cognitive impairment in Alzheimer's Disease: is there a gender effect? *Psychiatry Research*, 29, 169-175.
- Pagani, M., Lucini, D., Mela, G.S., Langewitz, W., Malliani, A. (1994). Sympathetic over-reactivity in subjects complaining of unexplained fatigue. *Clinical Science*, 87, 655-661.
- Palombo, V., Scurti, R., Muscari, A., Pudda, G.M., Di Iorio, A., Zito, M., Abate, G. (1997). Blood pressure and intellectual function in elderly subjects. *Age and Aging*, 26, 91-98.
- Pandav, R., Dodge, H.H., De Kosky, S.T., Ganguli, M. (2003). Blood pressure and cognitive impairment in India and the United States. *Archives of Neurology*, 60, 1123-1128.

- Panerelli, M., Holloway, C.D., Fraser, R., Connell, J.M., Ingram, M.C., Andreson, N.H., Kenyon, C.J. (1998). Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. *Journal of Clinical Endocrinology and Metabolism*, 83, 1846-1852.
- Papanicolaou, D.A., Petrides, J.S., Tsigos, C., Bina, S., Kalogeras, K.T., Wilder, R., Gold, P.W., Deuster, P.A., Chrousos, G.P. (1996). Exercise stimulates interleukin-6 secretion: inhibition by glucocorticoids and correlation with catecholamines. *American Journal of Physiology Endocrinology and Metabolism*, 271, 601-605.
- Pasceri, V., Willerson, J.T., Yeh, E.T. (2000). Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation*, 102, 2165-2168.
- Pearlin, L.I., Lieberman, M.A., Menaghan, E.G., Mullan, J.T. (1981). The stress process. *Journal of Health and Social Behavior*, 22, 337-356.
- Pearlin, L.I., McKean Skaff, M. (1997). Stress and the life course: a paradigmatic approach. *The Gerontologist*, 36, 239-247.
- Peng, C.K., Havlin, S., Hausdorff, J.M., Mietus, J.E., Stanley, H.E., Goldberger, A.L. (1995). Fractal mechanisms and heart rate dynamics. Long-range correlations and their breakdown with disease. *Journal of Electrocardiology*, 28, 59-65.
- Pereira, A.C., Floriano, M.S., Mota, G.F., Cunha, R.S., Herkenhoff, F.L., Mill, J.G., Krieger, J.E. (2003). Beta2 adrenoceptor functional gene variants, obesity, and blood pressure levels interactions in the general population. *Hypertension*, 42, 685-692.
- Perini, C., Muller, F.B., Buhler, F.R. (1988). Suppressed aggression and hyperdynamic cardiovascular regulation in normotensive offspring of essential hypertensive parents. *Journal of Cardiovascular Pharmacology*, 12, S130-S133.
- Perini, C., Muller, F.B., Rauchfleisch, U., Battegay, R., Hobi, V., Buhler, F.R. (1990). Psychosomatic factors in borderline hypertensive subjects and offspring of hypertensive parents. *Hypertension*, 16, 627-634.
- Perski, A., Olsson, G., Landou, C., De Faire, U., Theorell, T., Hamsten, A. (1992). Minimum heart rate and coronary atherosclerosis: independent relations to global severity and rate of progression of angiographic lesions in men with myocardial infarction at a young age. *American Heart Journal*, 123, 609-616.
- Petersen, R.C., Doddy, R., Kurz, A., Mohs, R.C., Morris, J.C., Rabins, P.V., Ritchie, K., Rosser, M., Thal, L., Winblad, B. (2001). Current concepts in mild cognitive impairment. *Archives of Neurology*, 58, 1985-1992.
- Petrovitch, H., White, L.R., Izmirliam, G., Ross, G.W., Havlik, R.J., Markesbery, W., Nelson, J., Davis, D.G., Hardman, J., Foley, D.J., Launer, L.J. (2000). Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: the HAAS. *Neurobiology of Aging*, 21, 57-62.
- Pickering, T.G. (1991). *Ambulatory Monitoring and Blood Pressure Variability*. Philadelphia; Science Press.
- Pickering, T.G., Gerin, W. (1990). Cardiovascular reactivity in the laboratory and the role of behavioral factors in hypertension: a critical review. *Annals of Behavioral Medicine*, 12, 3-16.



- Pickering, T.G., Gerin, W. (1992). Does cardiovascular reactivity have pathogenic significance in hypertensive patients? In Johnson, E.H., Gentry, W.D., Julius, S. (Eds.). *Personality, Elevated Blood Pressure, and Essential Hypertension*. Washington; Hemisphere.
- Pierce, T.W., Elias, M.F. (1993). Cognitive function and cardiovascular responsivity in subjects with a parental history of hypertension. *Journal of Behavioral Medicine*, 16, 277-294.
- Pierce, T.W., Grim, R.D., King, J.S. (2005). Cardiovascular reactivity and family history of hypertension: a meta analysis. *Psychophysiology*, 42, 125-131.
- Pincus, S.M., Cummins, T.R., Haddad, G.G. (1993). Heart rate in normal and aborted-SIDS infants. *American Journal of Physiology*, 264, 638-646.
- Piran, N., Kennedy, S., Garfinkle, P.E., Owens, M. (1985). Affective disturbances in eating disorders. *Journal of Nervous and Mental Disorders*, 173, 395-400.
- Poli, V., Balena, R., Fattori, E., Markatos, A., Yamamoto, M., Tanaka, H., Ciliberto, G., Rodan, G.A., Costantini, F. (1994). Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO Journal*, 13, 1189-1196.
- Pollack, M. (1994). Heart rate reactivity to laboratory tasks and in two daily life settings. *Psychosomatic Medicine*, 56, 271-276.
- Pollmächer, T., Haack, M., Schuld, A., Reichenberg, A., Yirmiya, Z. (2002). Low levels of circulating inflammatory cytokines - do they affect human brain functions? *Brain Behavior and Immunity*, 16, 525-532.
- Porter, N.M., Landfield, P.W. (2004). Stress hormones and brain aging: adding insult to injury? *Nature Neuroscience*, 1, 3-4.
- Porter, R.J., Barnett, N.N., Idely, A., McGuckin, E.A., O'Brien, T.O. (2002). Effects of hydrocortisone administration on cognitive function. *Journal of Psychopharmacology*, 16, 65-71.
- Pruessner, J.C., Hellhammer, D.H., Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol response to awakening. *Psychosomatic Medicine*, 61, 197-204.
- Pruessner, J.C., Wolf, O.T., Hellhammer, D.H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., Kirschbaum, C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sciences*, 61, 2539-2549.
- Pruessner, M., Hellhammer, D.K., Pruessner, J.C., Lupien, S. (2003). Self-reported depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. *Psychosomatic Medicine*, 65, 92-99.
- Radloff, L.S. (1977). The CES-D scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement*, 1, 385-401.
- Ralston, S.H., Russell, R.G., Gowen, M. (1990). Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. *Journal of Bone and Mineral Research*, 5, 983-988.
- Rapp, S., Brenes, G., Marsh, A.P. (2002). Memory enhancement training for older adults with mild cognitive impairment: a preliminary study. *Aging and Mental Health*, 6, 5-11.

- Rashid, M.N., Fuentes, F., Touchon, R.C., Wehner, P.S. (2003). Obesity and the risk of cardiovascular disease. *Preventative Cardiology*, 6, 42-47.
- Rask, E., Olsson, T., Soderberg, S., Andrew, R., Livingstone, D., Johnson, O., Walker, B.R. (2001). Tissue-specific dysregulation of cortisol metabolism in human obesity. *Journal of Clinical Endocrinology and Metabolism*, 86, 1418-1421.
- Ravogli, A., Trazzi, S., Villani, A., Mutti, E., Cuspidi, C., Sampierim, L., De Ambroggi, L., Parati, G., Zancetti, A., Mancina, G. (1990). Early 24 hour blood pressure elevation in normotensive subjects with parental hypertension. *Hypertension*, 16, 491-497.
- Rebuffe-Scrive, M., Bronnegard, M., Nilsson, A., Vedh, J., Gustafsson, J.A., Björntorp, P. (1990). Steroid hormone receptors in human adipose tissues. *Journal of Clinical Endocrinology and Metabolism*, 71, 1215-1219.
- Rechlin, T., Weis, M., Spitzer, A., Kaschka, W.P. (1994). Are affective disorders associated with alterations in heart rate variability? *Journal of Affective Disorders*, 32, 271-275.
- Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., Pollmächer, T. (2001). Cytokine-associated emotional and cognitive disturbances in humans. *Archives of General Psychiatry*, 58, 445-452.
- Reuben, D.B., Chen, A.I., Harris, T.B., Ferrucci, L., Rowe, J.W., Tracy, R.P., Seeman, T.E. (2002). Peripheral blood markers of inflammation predict mortality and functional decline in high-functioning community-dwelling. *Journal of the American Geriatric Society*, 50, 638-644.
- Reul, J.M.H.M., De Kloet, E.R. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology*, 117, 2505-2512.
- Ridker, P.M., Hennekens, C.H., Buring, J.E., Rifai, N. (2000a). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine*, 342, 836-843.
- Ridker, P.M., Rifai, N., Pfeiffer, M., Sacks, F., Lepage, S., Braunwald, E. (2000b). Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation*, 101, 2149-2153.
- Ridker, P.M., Rifai, N., Stamfer, M.J., Hennekens, C.H. (2000c). Plasma concentration of interleukin-6 and risk of future myocardial infarction among apparently healthy men. *Circulation*, 101, 1767-1772.
- Riese, H., Groot, P.F.C., Van Den Berg, M., Kupper, N.H.M., Magnee, E.H.B., Rohaan, E.J., Vrijkotte, T.G.M., Willemsen, G., De Geus, E.J.C. (2003). Large-scale ensemble averaging of ambulatory impedance cardiograms. *Behavior Research methods, Instruments and Computers*, 35, 467-477.
- Rittenhouse, P.A., Bakkum, E.A., Levy, A.D., Li, Q., Carnes, M., Van De Kar, L.D. (1994). Evidence that ACTH serotonin is regulated by serotonin<sub>2A/2C</sub> (5-HT<sub>2A/2C</sub>) receptors. *Journal of Pharmacology*, 271, 1647-1655.
- Ritter, S., Ritter, R.C., Barnes, C.D. (1992). *Neuroanatomy and Physiology of Abdominal Vagal Afferents*, CRC, Annual Arboration,
- Roberts, A.D.L., Wessley, T., Chandler, T., Papadopoulos, A., Cleare, A.J. (2004). Salivary cortisol response to awakening in chronic fatigue syndrome. *British Journal of Psychiatry*, 184, 136-141.

- Rodin, J. (1986). Aging and health: effects of the sense of control. *Science*, 233, 1271-1276.
- Roodman, G.D. (1999). Cell biology of the osteoclast. *Experimental Hematology*, 27, 1229-1241.
- Roosendaal, B. (2000). Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology*, 25, 213-238.
- Roosendaal, B., Brunson, K.L., Holloway, B.L., McGaugh, J.L., Baram, T.Z. (2002). Involvement of stress-released corticotrophin-releasing hormone in the basolateral amygdala in regulating memory consolidation. *Proceedings of the National Academy of Science*, 99, 13908-13913.
- Rosenbaum, D.S., Jackson, L.E., Smith, J.M., Garan, H., Ruskin, J.N., Cohen, R.J. (1994). Electrical alterants in heart rate variability to ventricular arrhythmia. *New England Journal of Medicine*, 330, 235-241.
- Rosenman, R.H., Hjemdahl, P. (1991). Is there a causal relationship of anxiety, stress or cardiovascular reactivity to hypertension? *Stress Medicine*, 7, 152-157.
- Rosmond, R., Bouchard, C., Björntorp, P. (2002a). Allelic variants in the GABA<sub>A</sub> $\alpha$ 6 receptor subunit gene (*GABRA6*) is associated with abdominal obesity and cortisol secretion. *International Journal of Obesity*, 26, 928-938.
- Rosmond, R., Bouchard, C., Björntorp, P. (2002b). Increased abdominal obesity in subjects with a mutation in the 5-HT<sub>2A</sub> receptor gene promoter. *New York Academy of Sciences*, 967, 571-575.
- Rosmond, R., Chagnon, Y.C., Holm, G., Chagnon, M., Perusse, L., Lindell, K., Carlsson, B., Bouchard, C., Björntorp, P. (2000). A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis. *Obesity Research*, 8, 211-218.
- Rosmond, R., Dallman, M.F., Björntorp, P. (1998). Stress-related cortisol secretion in men: relationship with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *Journal of Clinical Endocrinology and Metabolism*, 83, 1853-1859.
- Ross, C.E., Huber, J. (1985). Hardship and depression. *Journal of Health and Social Behavior*, 26, 312-327.
- Ross, R. (1999). Atherosclerosis - and inflammatory disease. *New England Journal of Medicine*, 340, 115-126.
- Rowe, J.W., Kahn, R.L. (1998). *Successful Aging*. New York; Patheon Books.
- Ruediger, H., Seibt, R., Scheuch, K., Krause, M., Alam, S. (2004). Sympathetic and parasympathetic activation in heart rate variability in male hypertensive patients under mental stress. *Journal of Human Hypertension*, 18, 307-315.
- Rusting, C.L., Nolen-Hoeksema, S. (1998). Regulating responses to anger: effects of rumination and distraction on angry mood. *Journal of Personality & Social Psychology*, 74, 790-803.
- Rutledge, T., Linden, W., Paul, D. (2000). Cardiovascular recovery from acute laboratory stress: reliability and concurrent validity. *Psychosomatic Medicine*, 62, 648-654.

- Ryan, C., Butters, N. (1986). The neuropsychology of alcoholism. In Wedding, D., Hortob, A.M., Webster, J. (Eds.). *The Neuropsychology Handbook*. New York; Springer.
- Ryan, R. (2002). Prevalence of treated hypertension in general practice in England and Wales, 1994 to 1998. *Health Statistics Quarterly*, 16, 14-18.
- Sapolsky, R.M. (1992). *Stress, the Aging Brain, and the Mechanisms of Neuron Death*. Cambridge, MA; MIT Press.
- Sapolsky, R.M. (2000). Stress hormones: good and bad. *Neurobiology of Disease*, 7, 540-542.
- Sapolsky, R.M., Krey, L.C., McEwen, B. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine Reviews*, 7, 284-301.
- Saul, J.P., Arai, Y., Berger, R.D., Lilly, L.S., Colucci, W.S., Cohen, R.J. (1991). Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. *American Journal of Cardiology*, 61, 1292-1299.
- Sauro, M.D., Jorgensen, R.S., Pedlow, C.T. (2003). Stress, glucocorticoids and memory: a meta-analytic review. *Stress*, 6, 235-245.
- Sausen, K.P., Lovallo, W.R., Wilson, M.F. (1991). Heart rate reactivity, behavior pattern, and parental hypertension as predictors of cardiovascular activity during cognitive challenge. *Psychophysiology*, 28, 639-647.
- Schacter, D.L. (1997). The cognitive neuroscience of memory: perspectives from neuroimaging research. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences*, 352, 1689-1695.
- Schaie, K.W., Blazer, D.G., House, J.S. (1992). *Aging, Health Behaviors and Health Outcomes*. Hillsdale, New Jersey; Erlbaum.
- Scheier, M.F., Carver, C.S. (1985). Optimism, coping, and health: Assessment and implications of generalized outcome expectancies. *Health Psychology*, 4, 219-247.
- Schlotz, W., Hellhammer, J., Schulz, P., Stone, A.A. (2004). Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response. *Psychosomatic Medicine*, 66, 207-214.
- Schneider, G.M., Jacobs, D.W., Gevirtz, R.N., O'Connor, D.T. (2003). Cardiovascular haemodynamic response to repeated mental stress in normotensive subjects at genetic risk of hypertension: evidence of enhanced adaptation, and delayed recovery. *Journal of Human Hypertension*, 17, 829-840.
- Schneiderman, N., Gellman, M., Peckerman, A., Hurwitz, B., Saab, P., Llabre, M., Ironson, G., Durel, L., Skyler, J., McCabe, P.M. (2000). Cardiovascular reactivity as an indicator of risk for future hypertension. In McCabe, P.M., Schneiderman, N., Field, T., Wallace, A.R. (Eds.). *Stress, Coping and Cardiovascular Disease*. Mahwah, NJ; Lawrence Erlbaum Associates.
- Schulkin, J. (2003). Allostasis a neural behavioral perspective. *Hormones and Behavior*, 43, 21-27.
- Schulz, P., Kirschbaum, C., Prussner, J., Hellhammer, D.H. (1998). Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Medicine*, 14, 91-97.

- Schwartz, A.R., Gerin, W., Davidson, K.W., Pickering, T.G., Brosschot, J.F., Thayer, J.F., Christenfeld, N., Linden, W. (2003). Toward a causal model of cardiovascular responses to stress and the development of cardiovascular disease. *Psychosomatic Medicine*, 65, 22-35.
- Schwerdtfeger, A., Schmukle, S.C., Egloff, B. (2005). Interactive effects of avoidant coping and parental hypertension on rate pressure product reactivity in women. *Annals of Behavioral Medicine*, 29, 106-115.
- Seeman, T., Berkman, L.F., Gulanski, B.I., Robbins, R.J., Greenspan, S.L., Charpentier, P.A., Rowe, J.W. (1995). Self-esteem and neuroendocrine response to challenge: MacArthur studies of successful aging. *Journal of Psychiatric Research*, 39, 69-84.
- Seeman, T.E., Crimmins, E.M., Huang, M.-H., Singer, B., Bucur, A., Gruenewald, T.L., Berkman, L.F., Reuben, D.B. (2004). Cumulative biological risk and socio-economic differences in mortality: MacArthur Studies of Successful Aging. *Social Science and Medicine*, 58, 1985-1997.
- Seeman, T.E., McAvay, G., Albert, M., Merrill, S., Rodin, J. (1996). Self efficacy beliefs and change in cognitive performance: MacArthur Studies of Successful Aging. *Psychology and Aging*, 11, 538-551.
- Seeman, T.E., McEwen, B.S., Singer, B.H., Albert, M.S., Rowe, J.W. (1997a). Increase in urinary cortisol excretion and memory declines: MacArthur Studies of Successful Aging. *Journal of Clinical Endocrinology and Metabolism*, 82, 2458-2465.
- Seeman, T.E., Robbins, R.J. (1994). Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocrine Reviews*, 15, 233-261.
- Seeman, T.E., Singer, B., Wilkinson, C.W., McEwen, B. (2001). Gender differences in age-related changes in HPA axis reactivity. *Psychoneuroendocrinology*, 26, 225-240.
- Seeman, T.E., Singer, B.H., Rowe, J.W., Horwitz, R.I., McEwen, B.S. (1997b). Price of adaptation – allostatic load and its health consequences. *Archives of Internal Medicine*, 157, 2259-2268.
- Segestrom, S.C., Miller, G.E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychological Bulletin*, 130, 601-630.
- Selye, H. (1956). *The stress of life*. Toronto: McGraw-Hill
- Semenchuk, E.M., Larkin, K.T. (1993). Behavioral and cardiovascular responses to interpersonal challenges among male offspring of essential hypertensives. *Health Psychology*, 12, 416-419.
- Sgoifo, A., De Boer, S.F., Haller, J., Koolhaus, J.M. (1996). Individual differences in plasma catecholamines and corticosterone stress responses of wild type rats. *Physiology and Behaviour*, 60, 1403-1407.
- Shapiro, D., Goldman, I.B., Jamner, L.D. (1995). Effects of anger / hostility, defensiveness, gender, and family history of hypertension on cardiovascular reactivity. *Psychophysiology*, 32, 425-435.
- Sharpley, C.E. (1998). Psychosocial stress-induced heart rate reactivity and atherogenesis: cause or correlation. *Journal of Behavioral Medicine*, 21, 411-432.

- Sheffield, D., Kittayaphong, R., Cascio, W.E., Light, K.C., Golden, R.N., Finkel, J.B., Koch, G.G., Sheps, D. (1998). Heart rate variability at rest and during mental stress in patients with coronary artery disease: differences in patients with high and low depression scores. *International Journal of Behavioral Medicine*, 5, 31-47.
- Sherwood, A., Allen, M.T., Fahrenberg, J., Kelsey, R.M., Lovallo, W.R., Van Dooren, L.J. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology*, 27, 1-23.
- Shigematsu, Y., Hamada, M., Kokubu, T.J. (1988). Significance of systolic time intervals in predicting prognosis of primary pulmonary hypertension. *Cardiology*, 18, 1109-1114.
- Silberberg, J., Fryer, J., Wlodarczyk, J., Robertson, R., Dear, K. (1999). Comparison of family history measures used to identify high risk of coronary heart disease. *Genetic Epidemiology*, 16, 344-355.
- Simpson, D.M., Wicks, R. (1988). Spectral analysis of heart rate indicates reduced baroreceptor-related heart rate variability in elderly persons. *Journal of Gerontology*, 43, 21-24.
- Singh, J.P., Larson, M.G., Manolio, T.A., O'Donnell, C.J., Lauer, M., Evans, J.C., Levy, D. (1999). Blood pressure responses during treadmill testing as a risk factor for new-onset hypertension. *Circulation*, 99, 1831-1836.
- Singh, J.P., Larson, M.G., Tsuji, H., Evans, J.C., O'Donnell, C.J., Levy, D. (1998). Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension*, 32, 293-297.
- Singh-Manoux, A., Adler, N.E., Marmot, M. (2003). Subjective social status: its determinants and its association with measures of ill health in the Whitehall II study. *Social Science and Medicine*, 56, 1321-1333.
- Sloviter, R.S., Valiquette, G., Abrams, G.M., Ronk, E.C., Sollas, A.L., Paul, L.A., Neubort, S. (1989). Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science*, 243, 535-538.
- Smith, A., Thomas, M., Whitely, H. (2000). Effects of upper respiratory tract illness on mood and performance over the working day. *Ergonomics*, 43, 752-763.
- Snieder, H., Dong, Y., Barbeau, P., Harshfield, G.A., Dalageorgou, C., Zhu, H., Carter, N.D., Treiber, F.A. (2002).  $\beta$ 2-adrenergic receptor gene and resting hemodynamics in European and African American youth. *American Journal of Hypertension*, 15, 973-979.
- Snöderlund, h., Nymberg, L., Adolfsson, R., Nilsson, L.-G., Launer, L.J. (2003). High prevalence of white matter hyperintensities in normal aging: relation to blood pressure and cognition. *Cortex*, 39, 1093-1105.
- Squire, L.R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological Review*, 99, 195-231.
- Stamler, J., Pick, R., Katz, L. (1954). Effects of cortisone, hydrocortisone and corticotrophin on lipemia, glycemia and atherogenesis in cholesterol fed-chicks. *Circulation*, 10, 237-246.

- Starkman, M.N., Gerbarski, S.S., Berent, S., Schteingart, D.E. (1992). Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's Syndrome. *Biological Psychiatry*, 32, 756-765.
- Starkman, M.N., Giordani, B., Gebarski, S.S., Berent, S., Schork, M.A., Schteingart, D.E. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry*, 46, 1595-1602.
- Starkman, M.N., Giordani, B., Gebarski, S.S., Schteingart, D.E. (2003). Improvement in learning associated with increase in hippocampal formation volume. *Biological Psychiatry*, 53, 233-238.
- Starkman, M.N., Schteingart, D.E. (1981). Neuropsychiatric manifestations of patients with Cushing's syndrome. Relationship to cortisol and adrenocorticotrophic hormone levels. *Archives of Internal Medicine*, 141, 215-219.
- Starkman, M.N., Schteingart, D.E., Schork, M.A. (1986). Cushing's syndrome after treatment; changes in cortisol and ACTH levels, and amelioration of the depressive syndrome. *Psychiatry Research*, 19, 177-188.
- Steele, C.M. (1997). A threat in the air. How stereotypes shape intellectual identity and performance. *American Psychologist*, 52, 615-629.
- Steenland, K., Henley, J., Thun, M. (2002). All-cause and cause specific death rates by educational status for two million people in two American cancer society cohorts, 1959-1996. *American Journal of Epidemiology*, 156, 11-21.
- Stein, P.K., Kleiger, R.E. (1999). Insights from the study of heart rate variability. *Annual Review of Medicine*, 50, 249-261.
- Step toe, A., Ayers, S. (2004). Stress, health and illness. In Sutton, S., Baum, A., Johnston, M. (Eds.). *The SAGE Handbook of Health Psychology*. London; Sage.
- Step toe, A., Kunz-Ebrecht, S.R., Owen, N., Feldman, P.J., Willemsen, G., Kirschbaum, C., Marmot, M. (2003a). Socioeconomic status and stress-related responses over the working day. *Psychosomatic Medicine*, 65, 461-470.
- Step toe, A., Marmot, M. (2002). The role of psychobiological pathways in socio-economic inequalities in cardiovascular disease risk. *European Heart Journal*, 23, 13-25.
- Step toe, A., Moses, J., Edwards, S. (1990). Age-related differences in cardiovascular reactions to mental stress tests in women. *Health Psychology*, 9, 18-34.
- Step toe, A., Vögele, C. (1991). Methodology of mental stress testing in cardiovascular research. *Circulation*, 82, II-14-II-24.
- Step toe, A. (1997). Behaviour and blood pressure: implications for hypertension. In Mancia, G. (Ed.). *Handbook of Hypertension - Pathophysiology of Hypertension*. Amsterdam; Elsevier Science.
- Step toe, A., Brydon, L. (2005). Psychoneuroimmunology and coronary heart disease. In Vedhara, K., Irwin, M. (Eds.). *Human Psychoneuroimmunology*. Oxford, UK; Oxford University Press.
- Step toe, A., Cropley, M. (2000). Persistent high job demand and reactivity to mental stress predict future ambulatory blood pressure. *Journal of Hypertension*, 18, 581-586.

- Steptoe, A., Cropley, M., Griffith, J., Joeke, K. (1999). The influence of abdominal obesity and chronic work stress on ambulatory blood pressure in men and women. *International Journal of Obesity*, 23, 1184-1191.
- Steptoe, A., Feldman, P.J. (2001). Neighborhood problems as sources of chronic stress: development of a measure of neighborhood problems, and associations with socioeconomic status and health. *Annals of Behavioral Medicine*, 23, 177-185.
- Steptoe, A., Kunz-Ebrecht, S., Owen, N. (2003b). Lack of association between depressive symptoms and markers of immune and vascular inflammation in middle-aged men and women. *Psychological Medicine*, 33, 667-674.
- Steptoe, A., Marmot, M. (2003). The burden of psychosocial adversity and vulnerability in middle-age: associations with biobehavioural risk factors and quality of life. *Psychosomatic Medicine*, 65, 1029-1037.
- Steptoe, A., Owen, N., Kunz-Ebrecht, S., Mohamed-Ali, V. (2002). Inflammatory cytokines, socioeconomic status, and acute stress responsivity. *Brain Behavior and Immunity*, 16, 774-784.
- Steptoe, A., Tavazzi, L. (1996). The mind and the heart. In Poole-Wilson, P.A. (Ed.). *Diseases of the Heart*. London; W.B. Saunders Co.
- Steptoe, A., Wardle, J. (2005). Cardiovascular stress responsivity, body mass and abdominal obesity. *International Journal of Obesity*, -In Press.
- Steptoe, A., Willemsen, G., Owen, N., Flower, L., Mohamed-Ali, V. (2001). Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clinical Science*, 101, 185-192.
- Sterling, P., Eyer, J. (1988). A new paradigm to explain arousal pathology. In Fisher, S., Reason, J. (Eds.). *Handbook of Life Stress, Cognition and Health*. Oxford, UK; John Wiley & Sons.
- Sternberg, E.M. (1997). Neural-immune interactions in health and disease. *Journal of Clinical Investigation*, 100, 2641-2647.
- Stewart, J.C., France, C.R. (2001). Cardiovascular recovery from stress predicts longitudinal changes in blood pressure. *Biological Psychology*, 58, 105-120.
- Stewart, P.M., Boulton, A., Kumar, S., Clark, P.M.S., Shackleton, H.L. (1999). Cortisol metabolism in human obesity: impaired cortisone to cortisol conversion in subjects with central adiposity. *Journal of Clinical Endocrinology and Metabolism*, 84, 1022-1027.
- Stewart, R., Kim, J.-M., Shin, I.-S., Joon, J.-S. (2003). Education and the association between vascular risk factors and cognitive function: a cross-sectional study in older Koreans with cognitive impairment. *International Psychogeriatrics*, 15, 27-36.
- Stitt, J.T. (1990). Passage of immunomodulators across the blood-brain barrier. *Yale Journal of Biological Medicine*, 63, 121-131.
- Stone, A.A., Schwartz, J.E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D.H., Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. *Psychoneuroendocrinology*, 26, 295-306.



- Stoney, C.M., Matthews, K.A., McDonald, R.H., Johnson, C.A. (1988). Sex differences in lipid, lipoprotein, cardiovascular, and neuroendocrine responses to acute stress. *Psychophysiology*, 25, 645-656.
- Straub, R.H., Konecna, L., Hrach, S., Rothe, G., Kreutz, M., Scholmerich, J., Falk, W., Land, B. (1998). Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: possible link between endocrinosensescence and immunosenescence. *Journal of Clinical Endocrinology and Metabolism*, 83, 2012-2017.
- Straub, R.H., Miller, L.E., Scholmerich, J., Zietz, B. (2000). Cytokines and hormones as possible links between endocrinosensescence and immunosenescence. *Journal of Neuroimmunology*, 109, 10-15.
- Strike, P.C., Steptoe, A., Wardle, J. (2004). Mild acute inflammatory stimulation induces transient negative mood. *Journal of Psychosomatic Research*, 57, 189-194.
- Suchday, S., Carter, M.M., Ewart, C.K., Larkin, K.T., Desiderato, O. (2004). Anger cognitions and cardiovascular recovery following provocation. *Journal of Behavioral Medicine*, 27, 319-341.
- Swan, G.E., Carmelli, D., La Rue, A. (1996). Relationship between blood pressure during middle age and cognitive impairment in old age: The Western Collaborative Group Study. *Aging Neuropsychology and Cognition*, 3, 241-252.
- Swanick, G.R., Coen, R.F., Walsh, J.B., Coakley, D., Lawlor, B.A. (1996). The predictive value of hypothalamic-pituitary-adrenal axis dysfunction in Alzheimer's disease. *Biological Psychiatry*, 39, 976-978.
- Taaffe, D.R., Harris, T.B., Ferrucci, L., Rowe, J.W., Seeman, T.E. (2000). Cross-sectional and prospective relationships of interleukin-6 and C-Reactive protein with physical performance in elderly persons: MacArthur Study of Successful Aging. *Journal of Gerontology*, 55, 709-715.
- Tanji, J.L., Champlin, J.J., Wong, G.Y., Lew, E.Y., Brown, T.C., Amsterdam, E.A. (1989). Blood pressure recovery curves after submaximal exercise. *American Journal of Hypertension*, 2, 135-138.
- Tauchmanova, L., Rossi, R., Biondi, B., Pulcrano, M., Nuzzo, V., Palmieri, A.-E., Fazio, S., Lombardi, G. (2002). Patients with subclinical Cushing's Syndrome due to adrenal adenoma have increased cardiovascular risk. *Journal of Clinical Endocrinology and Metabolism*, 87, 4872-4878.
- Thompson, S.C., Sobolew-Shubin, A., Galbraith, M.E., Schwankovsky, L., Cruzen, D. (1993). Maintaining perceptions of control: finding perceived control in low-control circumstances. *Journal of Personality and Social Psychology*, 64, 293-304.
- Thompson, S.C., Spacapan, S. (1991). Perceptions of control in vulnerable populations. *Journal of Social Issues*, 74, 1-21.
- Tops, M., Van Der Pompe, G., Baas, D., Mulder, L.M., Den Boer, J.A., Meijman, T.F., Korff, J. (2003). Acute cortisol effects on immediate free recall and recognition of nouns on stimulus valence. *Psychophysiology*, 40, 167-173.
- Torpy, D.J., Papanicolaou, D.A., Lotsikas, A.J., Wilder, R.L., Chrousos, G.P., Pillemer, S.R. (2000). Responses of the sympathetic nervous system and the hypothalamic-pituitary-

- adrenal axis to interleukin-6: a pilot study in fibromyalgia. *Arthritis & Rheumatism*, 43, 872-880.
- Treiber, F.A., Davis, H., Musante, L., Raunika, R.A., Strong, W.B., McCaffrey, F., Meeks, M.C., Vandernoord, R. (1993). Ethnicity, gender, family history of myocardial infarction and hemodynamic responses to lab stressors in children. *Health Psychology*, 12, 6-15.
- Treiber, F.A., Karamak, T.W., Schneiderman, N., Sheffield, D., Kapuku, G., Taylor, T. (2003). Cardiovascular Reactivity and Development of Preclinical and Clinical Disease States. *Psychosomatic Medicine*, 65, 46-62.
- Treiber, F.A., Strong, W.B., Arensman, F.W., Forrest, T., Davis, H., Musante, L. (1991). Family history of myocardial infarction and hemodynamic responses to exercise in young black boys. *American journal of diseases of children*, 145, 1029-1033.
- Tsigos, C., Chrousos, G.P. (1996). Stress, endocrine manifestations, and diseases. In Cooper, G. (Ed.). *Handbook of Stress, Medicine and Health*. New York; CRC Press.
- Tulsky, D.S., Haaland, K.Y. (2001). Exploring the clinical utility of WAIS-III and WMS-III. *Journal of the International Neuropsychological Society*, 7, 860-862.
- Tulsky, D.S., Ledbetter, M.F. (2000). Updating to the WAIS-III and WMS-III: considerations for research and clinical practice. *Psychological Assessment*, 12, 253-262.
- Turnball, A.V., Rivier, C.L. (1999). Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiological Reviews*, 79, 1-71.
- Turner, J.R. (1994). *Cardiovascular Reactivity and Stress*. New York; Plenum Press.
- Turner, J.R., Hewitt, J.K. (1992). Twin studies in cardiovascular response to psychological challenge: a review and suggested future directions. *Annals of Behavioral Medicine*, 14, 12-20.
- Turner, J.R., Ward, M.M., Gellman, M.D., Johnston, D.W., Light, K.C., Van Doornen, L.J.P. (1994). The relationship between cardiovascular reactivity: mechanisms and pathways to cardiovascular disease. *Annals of Behavioral Medicine*, 16, 12-23.
- Turner, R.J., Marino, F. (1994). Social support and social structure: a descriptive epidemiology. *Journal of Health and Social Behavior*, 35, 193-212.
- Turner, R.J., Wheaton, B. (1995). Checklist measurement of stressful life events. In Cohen, S., Kessler, R.C., Gordon, L.U. (Eds.). *Measuring Stress: A Guide for Health and Social Scientists*. New York, NY, US: Oxford University Press.
- Tzourio, C., Dufouil, C., Ducimetiere, P., Alperovitch, A. (1999). Cognitive decline in individuals with high blood pressure: a longitudinal study in the elderly. *Neurology*, 53, 1948-1952.
- Uchino, B.N., Cacioppo, J.T., Malarkey, W., Glaser, R. (1995). Individual differences in cardiac sympathetic control predict endocrine and immune responses to acute psychological stress. *Journal of Personality and Social Psychology*, 69, 736-743.
- Ukkola, O., Rankinen, T., Weisnagel, S.J., Sun, G., Perusse, L., Chagnon, Y.C., Despres, J.P., Bouchard, C. (2000). Interactions among the alpha2-, beta2-, and beta3-adrenergic receptor genes and obesity-related phenotypes in the Quebec Family Study. *Metabolism*, 49, 1063-1070.

- Uden, A.L., Orth-Gomer, K., Eloffsson, S. (1991). Cardiovascular effects of social support in the work place: twenty-four hour ECG monitoring in men and women. *Psychosomatic Medicine*, 53, 50-60.
- Ursin, H. (1998). The psychology in psychoneuroendocrinology. *Psychoneuroendocrinology*, 23, 555-570.
- Ursin, H., Eriksen, H.R. (2004). The cognitive activation theory of stress. *Psychoneuroendocrinology*, 29, 567-592.
- Van Boxtel, M.P.J., Gaillard, C., Houx, J.P., Buntinx, F., De Leeuw, P.W., Jolles, J. (1997). Can the blood pressure predict cognitive task performance in a healthy population sample? *Journal of Hypertension*, 15, 1069-1076.
- Van Cauter, E., Leproult, R., Kupfer, D.J. (1996). Effect of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology and Metabolism*, 81, 2468-2473.
- Van Coevorden, A., Mockel, J., Laurent, E., Kerkhofs, M., L'Hermite-Balarioux, M., Descoster, C., Neve, P., Van Cauter, E. (1991). Neuroendocrine rhythms and sleep in aging men. *American Journal of Physiology*, 260, 651-661.
- Van Doornen, L.J.P., Knol, D.L., Willemsen, G., De Geus, E.J.C. (1994). The relationship between stress reactivity in the laboratory and in real-life: is reliability the limiting factor? *Journal of Psychophysiology*, 8, 297-304.
- Van Honk, J., Kessels, R.P.C., Putman, P., Jager, G., Koppescharr, H.P.F., Postma, A. (2003). Attentional modulated effects of cortisol and mood on memory for emotional faces in healthy young males. *Psychoneuroendocrinology*, 28, 941-948.
- Van Melle, J.P., De Jonge, P., Spukerman, T.A., Tussen, J.G.P., Ormel, J., Van Veldhuisen, D.J., Van Den Brink, R.H.S., Van Den Berg, M.P. (2004). Prognostic association of depression following myocardial infarction with mortality and cardiovascular events: a meta-analysis. *Psychosomatic Medicine*, 66, 814-822.
- Van Swieten, J.C., Geyskes, O.G., Derixm, M.A. (1991). Hypertension in the elderly is associated with white matter lesion and cognitive decline. *Annals of Neurology*, 30, 825-830.
- Vedhara, K., Cox, N.K.M., Wilcock, G.K., Perks, P., Hunt, M., Anderson, S., Lightman, S.L., Shanks, N.M. (1999). Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *Lancet*, 353, 627-631.
- Vicennati, V., Pasquali, R. (2000). Abnormalities of the hypothalamic-pituitary-adrenal axis in non-depressed women with abdominal obesity and relations with insulin resistance: evidence for a central peripheral alteration. *Journal of Clinical Endocrinology and Metabolism*, 85, 4093-4098.
- Vitaliano, P.P., Russo, J., Paulsen, V.M., Bailey, S.L. (1995). Cardiovascular recovery from laboratory stress: biopsychosocial concomitants in older adults. *Journal of Psychosomatic Research*, 39, 361-377.
- Vlacyiannopoulos, P.G., Kanellopoulos, P.G., Ioannidis, J.P., Tektonidou, M.G., Mastorakou, I., Moutsopoulos, H.M. (2003). Atherosclerosis in premenopausal women with antiphospholipid syndrome and systemic lupus erythematosus: a controlled study. *Rheumatology*, 42, 645-651.

- Vögele, C., Steptoe, A. (1992). Emotional coping and tonic blood pressure as determinants of cardiovascular responses to mental stress. *Journal of Hypertension*, 10, 1087.
- Vögele, C., Steptoe, A. (1993). Anger inhibition and family history as modulators of cardiovascular responses to mental stress in adolescent boys. *Journal of Psychosomatic Research*, 37, 503-514.
- Vollmer-Conna, U. (2001). Acute sickness behavior: an immune system-to-brain communication? *Psychological Medicine*, 31, 761-767.
- Vollmer-Conna, U., Fazou, C., Cameron, B., Li, H., Brennen, C., Luck, L., Davenport, T., Wakefield, D., Hickie, I., Lloyd, A. (2004). Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behavior in humans. *Psychological Medicine*, 34, 1289-1297.
- Vollmer-Conna, U., Wakefield, D., Llyod, A., Hickie, I., Lemon, J., Bird, K.D., Westbrook, R.F. (1997). Cognitive deficits in patients suffering from chronic fatigue syndrome, acute infective illness or depression. *British Journal of Psychiatry*, 171, 377-381.
- Vrijkotte, T.G.M., Lorenz, J.P., Van Dooren, L.J., De Geus, E.J.C. (2004). Over commitment to work is associated with changes in cardiac sympathetic regulation. *Psychosomatic Medicine*, 66, 656-663.
- Wadsworth, M.E.J. (1986). Serious illness in childhood and its association with later-life achievement. In Wilkinson, R.G. (Ed.). *Class and Health. Research and Longitudinal Data*. New York; Tavistock Publications.
- Waldstein, S.R. (2003). The relation of hypertension to cognitive function. *Current Directions in Psychological Science*, 12, 9-12.
- Waldstein, S.R., Burns, H.O., Toth, M.J., Poehlman, E.T. (1999). Cardiovascular reactivity and central adiposity in older African Americans. *Health Psychology*, 18, 221-228.
- Waldstein, S.R., Elias, M.F. (2001). *Neuropsychology of Cardiovascular Disease*. Mahwah, NJ; Erlbaum.
- Waldstein, S.R., Jennings, J.R., Ryan, C.M., Muldoon, M.F., Shapiro, A.P. (1996). Hypertension and neuropsychological performance in men: interactive effects of age. *Health Psychology*, 15, 102-109.
- Waldstein, S.R., Ryan, C.M., Manuck, S.B., Parkinson, D.K., Bromet, E.J. (1991). Learning and memory function in men with untreated blood pressure elevation. *Journal of Consulting and Clinical Psychology*, 59, 513-517.
- Wallace, R.B., Lemke, J.H., Morris, M.C., Goodenberger, M., Kohout, F., Hinrichs, J.V. (1985). The relationship of free-recall memory to hypertension in the elderly. The Iowa 65+ rural health study. *Journal of Chronic Disorders*, 38, 475-481.
- Walsberg, G.E. (2003). How useful is energy balance as an overall index of stress in animals? *Hormones and Behavior*, 43, 16-17.
- Ward, P.A. (1995). Cytokines, inflammation, and autoimmune diseases. *Hospital Practice*, 30, 35-41.
- Watkins, L.R., Maier, S.F., Goehler, L.E. (1995). Cytokine-to-brain communication: a review and analysis of alternative mechanisms. *Life Sciences*, 57, 1011-1026.

- Watt, G.C.M., Foy, C.J.W., Holton, D.W., Edwards, H.E. (1991). Prediction of high blood pressure in young people: the limited usefulness of parental blood pressure in young people. *Journal of Hypertension*, 9, 55-58.
- Weaver, J.D., Huang, M.-H., Albert, M.S., Harris, T., Rowe, J.W., Seeman, T.E. (2002). Interleukin-6 and risk of cognitive decline: MacArthur Studies of Successful Aging. *Neurology*, 59, 371-378.
- Weaver, J.U., Hitman, G.A., Kopelman, P.G. (1992). An association between a BCII restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. *Journal of Molecular Endocrinology*, 9, 295-300.
- Westley-Wise, V.J., Beard, J.R., Sladden, T.J., Dun, T.M., Simpson, J. (1996). Ross River virus infection on the north coast of New South Wales. *Australian and New Zealand Journal of Public Health*, 20, 876-892.
- Whelan, T.B., Schteingart, D.E., Starkman, M.N., Smith, A. (1980). Neuropsychological deficits in Cushing's Syndrome. *Journal of Nervous and Mental Disease*, 168, 753-757.
- Whitaker, R.C., Wright, J.A., Pepe, M.S., Seidel, K.D., Dietz, W.H. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *New England Journal of Medicine*, 337, 869-873.
- Wijbenga, J.A.M., Balk, A.H.M.M., Meij, S.H., Simoons, M.L., Malik, M. (1998). Heart rate variability index in congestive heart failure: relation to clinical variables and prognosis. *European Heart Journal*, 19, 1719-1724.
- Wilder, R.L. (1995). Neuroendocrine-immune system alterations and autoimmunity. *Annual Review of Immunology*, 13, 307-338.
- Wilkinson, R.G. (1999). Health, hierarchy, and social anxiety. *Annals of the New York Academy of Sciences*, 696, 48-63.
- Willemsen, G.H.M., De Geus, E.J.C., Klaver, C.H.A.M. (1996). Ambulatory monitoring of the impedance cardiogram. *Psychophysiology*, 33, 184-193.
- Williams, E., Magid, K., Steptoe, A. (2005). The impact of time of waking and concurrent subjective stress on cortisol response to awakening. *Psychoneuroendocrinology*, 30, 139-148.
- Williams, R.B., Marchuk, D.A., Gadde, K.M., Barefoot, J.C., Grichnik, K., Helms, M.J., Kuhn, C.N., Lewis, J.G., Scanberg, S.M., Stafford-Smith, M., Suarez, E.C., Clary, G.L., Svenson, I.K., Siegler, I.C. (2001a). Central nervous system serotonin function and cardiovascular responses to stress. *Psychosomatic Medicine*, 63, 300-305.
- Williams, R.R., Hunt, S.C., Heiss, G., Province, M.A., Bensen, J.T., Higgins, M., Chamberlain, R.M., Ware, J., Hopkins, P.N. (2001b). Usefulness of cardiovascular family history data for population-based preventative medicine and medical research (the Health Family Tree Study and the NHLBI Family Heart Study). *American Journal of Cardiology*, 87, 129-135.
- Wilson, R.S., Bennett, D.A., Mendes De Leon, C.F., Bienias, J.L., Morris, M.C., Evans, D.A. (2005). Distress proneness and cognitive decline in a population of older persons. *Psychoneuroendocrinology*, 30, 11-17.

- Wolf, O.T., Convit, A., McHugh, P.F., Kandil, E., Thorn, E.L., De Santi, S., McEwen, B.S., De Leon, M.J. (2001a). Cortisol differentially affects memory in young and elderly men. *Behavioral Neuroscience*, 115, 1002-1011.
- Wolf, O.T., Convit, A., Thorn, E., De Leon, M. (2002). Salivary cortisol day profiles in elderly with mild cognitive impairment. *Psychoneuroendocrinology*, 27, 777-789.
- Wolf, O.T., Schommer, N.C., Hellhammer, D.H., McEwen, B.S., Kirschbaum, C. (2001b). The relationship between stress induced cortisol levels and memory differs between men and women. *Psychoneuroendocrinology*, 26, 711-720.
- Wolkowitz, O.M., Reus, V.I., Weingartner, H., Thompson, K., Breier, A., Doran, A., Rubinow, D., Pickar, D. (1990). Cognitive effects of corticosteroids. *American Journal of Psychiatry*, 147, 1297-1303.
- Wong, C.K., Ho, C.Y., Ko, F.W.S., Chan, C.H.S., Ho, A.S.S., Hui, D.S.C., Lam, C.W.K. (2001). Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and the Th cytokines (INF-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clinical and Experimental Immunology*, 125, 177-183.
- Wood, D.L., Sheps, S.G., Elveback, L.R., Schirger, A. (1984). Cold pressor test as a predictor of hypertension. *Hypertension*, 6, 301-306.
- Workman, M.L. (2000). Immune mechanisms in rheumatism disease. *Nursing Clinics of North America*, 35, 175-188.
- Wüst, S., Van Rossum, E.F.C., Federenko, I.S., Koper, J.W., Kumsta, R., Hellhammer, D.H. (2004). Common polymorphisms in the glucocorticoid receptor gene are associated with adrenocortical responses to psychosocial stress. *Journal of Clinical Endocrinology and Metabolism*, 89, 565-573.
- Wüst, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N.C., Kirschbaum, C. (2000). The cortisol awakening response: normal values and confounds. *Noise and Health*, 77, 77-85.
- Yeragani, V.K., Mallavarapu, M., Radhakrishna, R.K.A., Tancer, M., Uhde, T. (2004). Linear and nonlinear measures of blood pressure variability: Increased chaos of blood pressure time series in patients with panic disorder. *Depression and Anxiety*, 19, 85-95.
- Yirmiya, R. (1997). Behavioral and Psychological effects of immune activation: implications for 'depression due to general medical condition'. *Current Opinion in Psychiatry*, 10, 470-476.
- Yirmiya, R., Weidenfeld, J., Pollak, Y., Morag, M., Morag, A., Avitsur, R., Barak, O., Reichenberg, A., Cohen, E., Shavit, Y., Ovadia, H. (1999). Cytokines, "depression due to a general medical condition," and antidepressant drugs. *Advances in Experimental Medicine & Biology*, 461, 283-316.
- Yudkin, J.S., Stehouwer, C.D., Emeis, J.J., Coppel, S.W. (1999). C-reactive protein in healthy subjects: association with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue. *Arteriosclerosis, Thrombosis & Vascular Biology*, 19, 972-978.
- Zajonc, R.B. (1984). On the primacy of affect. *American Psychologist*, 39, 117-123.

## Appendix I: Recruitment Letter (Study 1)

*Dr G Graham*  
*Dr S Seyan*  
*Dr J M Justice*  
*Dr D Rozewicz*  
*Dr M Malone*  
*Dr K Paul*  
*Dr D Goldwater*

*Simpson House*  
*Medical Centre*

### AGE AND HEALTH STUDY

Dear

We are writing to ask if you would be willing to help us in a piece of work on physical health after the age of 65. We want to examine aspects of daily life that might contribute to better physical health for people in a study that is being carried out with colleagues at UCL Medical School which is funded by the Medical Research Council.

We want to understand how factors such as lifestyle influence health by asking people in your age group about their relationships, lifestyles, and attitudes toward life, but we also want to take some biological measures like blood pressure and heart rate at Simpson House Medical Centre.

We would like to ask you to come to Simpson House for a visit estimated to last between 90-120 minutes. Before the visit we will send you some information and a questionnaire to complete at home.

The findings from this study will not only help us to understand the relationship between psychological factors and health in later life, but also may be used to improve policy related to health and social care for older people.

If you are interested in taking part in this study, please fill in and return the enclosed card to UCL in the FREEPOST envelope (no stamp needed). If you have any questions or would like more information before deciding, please phone Liz Cort or Lindsey Emmerson on 020 7679 5634.

If you do not want to participate in the Age and Health Study, it would greatly help us if you could indicate this on the enclosed card and return it in the FREEPOST envelope.

Yours sincerely

**Dr. Daniel E. Goldwater MB ChB DCH MRCP**

## Appendix II: Information Sheet (Study 1)

### AGE AND HEALTH RESEARCH PROJECT

#### Information Sheet

Doctors and medical researchers recognize that the way we live our daily lives can influence our health. As we grow older, there are changes in our social relationships and in our day to day activities and life styles which may influence our physical health. We are carrying out a research project to find out whether these changes have an influence on biological processes related to disability and disease, as well as physical health. We are therefore inviting a number of patients registered with the Simpson House Medical Centre to take part in this research study.

There are two parts to this study:

- 1) A member of the research staff working on the study will interview you at the Simpson House Medical Centre about aspects of your daily life and your well-being. This interview will involve questions about social relationships, depressive symptoms and recent life events you may have experienced. The research assistant will take physical measurements such as body weight. The researcher will also assess your motor control and walking. You will be asked to provide some saliva samples throughout the interview. The session will take about 2 hours. Before your appointment we give you a set of questionnaires to complete at home. These questionnaires ask about your life style, quality of life, social support, and attitudes and feelings toward life.
- 2) On a separate day we want to take a number of saliva that are related to health and well-being. We will give you a set of tubes, each of which contains a small roll of cotton wool. Every couple of hours over the day, we want you to put a roll of cotton wool in your mouth for two minutes, then return it to the tube. We will give you an envelope to post them back to us.

Your participation is very important to us. All the information we collect will be completely confidential, and will be used for research purposes only. If there are any abnormal results, your GP will discuss them with you following the visit. Please remember, you do not have to take part in this study if you do not want to. If you decide to take part, you can withdraw at any time without having to give a reason. Your decision whether to take part or not will not affect your care or management in any way.

*All proposals for research using human subjects are reviewed by an ethics committee before they can proceed. This proposal was reviewed by Camden & Islington Local Research Ethics Committee.*



## Appendix III: Consent Form

# Royal Free and University College Medical School

## UNIVERSITY COLLEGE LONDON

DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH

Gower Street Campus  
1-19 Torrington Place

London WC1E 6BT



### CONSENT FORM (Confidential)

Participant Number.....

#### Title of project:

Name of Researchers: Professor Andrew Steptoe, Caroline Wright, Bev Murray, Katie O'Donnell  
& Dr Lena Brydon

#### Please initial box

1. I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐

Name of volunteer	Date	Signature

Researcher	Date	Signature

**Appendix IV: List of Word Pairs (VPA 1 & VPA 2)**

Truck-Arrow

Insect-Acorn

Reptile-Clown

Bank-Cartoon

Star-Ladder

Raccoon Paper

Rose-Bag

Elephant-Glass

## Appendix V: VPA 1 Instruction Sheet

### Verbal Paired Associates 1

***'I am going to say a word and then say another word that goes with it. I will say a whole list of words like that. Listen carefully because when I have finished I will say the first word, and I want you to tell me the word that goes with it. For example, if the word pairs were Fruit-West, Gold-Walk, then when I say the word Fruit, you would answer (pause) West. When I say the word Gold, you would answer (pause) Walk. Do you understand?'***

If the examinee begins to read the word pairs as you are reading, stop and instruct him or her to wait until you are finished. If the examinee does not understand the directions, you may repeat them, paraphrasing where necessary.

Read the word pairs at a rate of one pair of words every 3 seconds; that is, the words are spoken 1 second apart, with 2 seconds separating the pairs.

When you are sure the examinee understands the directions, say ***Now listen carefully to the list of word pairs as I read them.*** Read List A from the record form.

After reading List A, pause for 5 seconds and present Recall A.

Ask ***Which word goes with \_\_\_\_\_ ?***

Read the first word of each pair. Allow a maximum of 5 seconds for the examinee's response. Record the examinee's response.

If the examinee responds correctly, say ***That's right*** and proceed to the next word in the Recall List.

If the examinee responds incorrectly, say ***No, \_\_\_\_\_ goes with \_\_\_\_\_*** and provide the examinee with the correct response. Then proceed to the next word in the Recall List. If the examinee gives no response within 5 seconds, score the item

as 0, provide the examinee with the correct response, and proceed to the next word in the Recall List.

After completing List A, pause for 5 seconds. Then say ***Now I will read the same list again, except with the word pairs in a different order. Listen carefully.***

Read List B from the Record Form.

After reading List B, pause for 5 seconds and present Recall B, using the same procedures as with Recall A. You may repeat the question '***Which word goes with \_\_\_\_\_?***' if necessary.

Continue with List C and Recall C, followed by List D and Recall D.

When the subset is completed, say ***Later on I will ask you to recall these word pairs again, so try to remember them.***

## Appendix VI: VPA 2 Instruction Sheet

### Verbal Paired Associates II

#### Recall

***'Remember the word pairs that you learned earlier? I told you a word and gave you another word that went with it. I want you to recall as many word pairs as you can remember, one more time. I will say the first word of the pair, and you say the word that goes with it. Ready?'***

Present the Recall section as follows:

***Which word goes with \_\_\_\_\_? (Read the first word of the pair).***

Allow approximately 10 seconds for the examinee to respond. Record the examinee's response. Do not tell the examinee if his or her response is correct or incorrect. Continue in this manner until all recall items have been administered.

After completing Recall, proceed with recognition.

#### Recognition

***'I am going to read a list of word pairs. Listen carefully. After I read a word pair, I want you to say Yes if it was one of the pairs I asked you to remember earlier or No if it is a new word pair. Some word pairs may be said more than once. Do you understand?'***

If the examinee does not understand the instructions, you may repeat them, paraphrasing where necessary. When you are sure the examinee understands the directions, start by reading the first word pair of the Recognition list.

Read each word pair (Items 1-24) in the order shown on the Record Form and record the examinee's responses.

## Appendix VII: Matrix Reasoning Instruction Sheet

### Matrix Reasoning

To introduce the subset say:

***'I am going to show you some pictures. For each picture, there is a part missing. Look at all aspects of each picture carefully and choose the missing part from the five choices.'***

For sample items A-C, provide the teaching included in the item instructions if the examinee responds incorrectly.

Item instructions:

Item A: Turn to item A in the booklet. Place it in front of the examinee and say:

***For example, tell me which of these pictures*** (point to the response choices) ***should go here*** (point to the question mark). ***Make sure you carefully look at the picture on top and the response choices below before making your selection. If you think there is more than one correct answer to the problem, choose the best one. Remember, you are to choose the one that best completes the pattern.***

If the examinee responds correctly (response 2), proceed to item B.

If the examinee responds incorrectly, say:

***For this item, the missing part should complete the pattern by making the picture the same colour as the pattern because the squares are all yellow.***

Item B: Turn to item B and say:

***Now tell me which of these picture*** (point to the response choices) ***should go here*** (point to the question mark). ***Again, make sure you carefully look at the picture on top and at the pictures below before choosing your answer. If you think there is more than one correct answer to the problem, choose the best one.***

If the examinee responds correctly (response 5), proceed to item C.

If the examinee responds incorrectly, say:

***There are a number of ways you can solve this problem. For instance, you can look at the pictures by separating them into two columns. Notice the pictures in the left column are the same*** (point at the two blue octagons).

***They are both the same shape, and they are both blue. Now look at the right column*** (point to the yellow circle and the question mark). ***One of these choices below*** (point to the response choices) ***will make the pictures on the right hand column the same as well. See, this choice here (response 5) would make the pictures in the right column both yellow circles.***

If the examinee does not understand or is confused about the reasoning behind the task, go over each step of the problem. If necessary, provide an alternative explanation to the problem (e.g. demonstrate how the problem can be solved by looking at rows).

Item C:

Turn to item C and say:

***Now tell me which of these pictures*** (point to the response choices) ***should go here*** (point to the question mark).

If the examinee responds correctly (response 4), proceed to item 4.

If the examinee responds incorrectly, say:

***All the pictures at the top are circles, and each large circle is followed by a*** (point to all the shapes in a sweeping motion) ***small one. Therefore, the small circle (response 4) is the best answer.***

Then proceed to item 4 in the stimulus booklet.

Items 1-26:

For each of items 1-3 (if administered) and each items 4-26 say:

***Now tell me which of these pictures*** (point to the response choices) ***should go here*** (point to the question mark).

No feedback or teaching should be given for items 1-3 and items 4-26. The examinee may make educated guesses, but instruct the examinee not to guess randomly.

## Appendix VIII: Medication Count and Chronic Illness Check Sheet

### Age and Health Study

#### Proforma to record medical history and current medication

**Date of session:**

**Participant ID number:**

**List current medication on reverse of this form unless detailed on chart below**

Condition	Yes/No (date)	Currently on medication yes/no and list	Comment (e.g. past history of taking medication, or possible related illness)
Taking NSAIDs			
High cholesterol (taking any anticholesterol meds?)			
Hormonal Problems (on HRT?)			
Hypertension (any medication?)			
On steroid therapy?			
Cardiovascular disease, angina, atherosclerosis			
CVA, central nervous disease, peripheral neuropathy			
Diabetes			
History of cancer			
Arthritis			
Asthma			
Allergies			
Psychiatric history or current MH problems			
Any current or history of depression			
Any recent vaccination or immunization (give type and dates within 3/12)			
Surgery in previous year			
Other main illness			

**Write any additional information overleaf**



## Appendix IX: Health Behaviour Questionnaire

### LIFESTYLE QUESTIONS

1. Do you smoke? (circle one response) YES NO

a) If 'Yes' how many of the following do you smoke?

Cigarettes \_\_\_\_\_ Cigars \_\_\_\_\_ Pipes \_\_\_\_\_

b) Have you ever smoked in the past? YES NO  
(circle one response)

If 'Yes' which year did you last smoke? \_\_\_\_\_

2. Do you drink alcohol? (circle one response) YES NO

a) If 'Yes', how many units per week on average do you drink?

(One unit = ½ pint of beer, 1 glass of wine or 1 measure of spirit)

\_\_\_\_\_ Units per week on average

3. How physically active are you? (circle one response)

Very physically active	Fairly physically active	Not very physically active	Not at all physically active
---------------------------	-----------------------------	-------------------------------	---------------------------------

4. Think back over the last 4 weeks about the sports or recreational activities that you have done.

How many times have you performed the following activities for at least 20 minutes during the last 4 weeks?

	Number of occasions activity performed for at least 20 minutes during last 4 weeks
<b>Example: Volleyball</b>	<b>5</b>
Swimming	
Running or Jogging	
Exercise of Keeping Fit	
Bicycling or Exercise Bike	
Tennis or Badminton	
Golf	
Fishing	
Darts	
Brisk Walking	
Bowls or Bowling	
Dancing	
Other Sports, Please specify: <hr/>	

## Appendix X: Task Impact Questionnaire

STUDY NAME:

Task Impact Questionnaire

Subject Code.....

Please answer the following questions by circling the number that best describes the way you felt during the task

1. How difficult did you find the task?

Not at all difficult						Very difficult
1	2	3	4	5	6	7

2. How involved in the task did you feel?

Not at all involved						Very involved
1	2	3	4	5	6	7

3. How well do you think you performed the task?

Not at all well						Very well
1	2	3	4	5	6	7

4. How stressed did you feel during the task?

Not at all stressed						Very stressed
1	2	3	4	5	6	7

5. How much in control of the task did you feel?

Not at all in control						Very in control
1	2	3	4	5	6	7

6. How relaxed did you feel during the task?

Not at all relaxed						Very relaxed
1	2	3	4	5	6	7

## Appendix XI: Rest Questionnaire

**STUDY NAME:**

**Rest Questionnaire**

**Subject Code.....**

**Please answer the following questions by circling the number that best describes the way you feel**

**7. How relaxed do you feel at the moment?**

<b>Not at all relaxed</b>						<b>Very relaxed</b>
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>

**8. How anxious do you feel at the moment?**

<b>Not at all anxious</b>						<b>Very anxious</b>
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>

**9. How stressed do you feel at the moment**

<b>Not at all stressed</b>						<b>Very stressed</b>
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>

## Appendix XII: Protocol (Study 1)

Date: \_\_\_\_\_ Time: \_\_\_\_\_ Study No: \_\_\_\_\_  
 M / F Age: \_\_\_\_\_ years Starting time: \_\_\_\_\_

Explain the procedure (roughly)

Consent form and collect main questionnaire

### Start 1. stopwatch (for saliva samples)

#### A) Structured Interview

1. How are you today? \_\_\_\_\_
2. Any unusual event during the last few days? No / Yes, if Yes \_\_\_\_\_  
 \_\_\_\_\_
3. Eat and drink today?  
 (breakfast/lunch) \_\_\_\_\_  
 \_\_\_\_\_
4. Sleep last night? Went to bed at: \_\_\_\_ Got up at: \_\_\_\_ Slept well/badly (circle)
5. Alcoholic drinks last night /  
 today? \_\_\_\_\_
6. Any medication tonight or  
 today? \_\_\_\_\_
7. Exercise last day,  
 today? \_\_\_\_\_

#### B) Physical Exercise

*Explain, why, use 2. stopwatch*

Stand up as often as you can in 20 seconds (without using your hands)

How often \_\_\_\_\_

Keep balance for at least 1 min: how long: \_\_\_\_\_ seconds

#### C) Physical Measures

Height \_\_\_\_\_ m Weight \_\_\_\_\_ kg  
 Hip measure \_\_\_\_\_ cm Waist measure \_\_\_\_\_ cm

*Always take left arm!* 1. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

*Explain straw-procedure: 'Swallow first and put your lips around the straw'.*

*If problems: put straw under tongue to stimulate saliva flow*

1. Saliva sample, time: \_\_\_\_\_

Give mood questionnaire **REST A**

### **Pulmonary Function**

*Switch on, insert mouthpiece, let participant breathe out as hard and long as possible write down all 4 values: F1 \_\_\_\_\_ VC \_\_\_\_\_ FER \_\_\_\_\_ PEF \_\_\_\_\_*

### **Impedance Monitoring**

*Attach AMS-electrodes front \_\_\_\_\_ back \_\_\_\_\_*

*Switch of lights, "close your eyes and relax"*

*Start 6 min AMS baseline, use 2. stopwatch*

*Press red button at beginning*

*After 6 min: 2. Saliva sample, time: \_\_\_\_\_*

*Press red button at end*

2. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

*After BP: 3. Saliva sample, time \_\_\_\_\_*

### **REST B**

**! STOP HERE** (if 'problems')

### **D) Cognitive functioning**

Verbal Paired Associates 1: **Read instructions**

*Press read button (AMS) and begin testing*

Fill in results:

List A	Recall A	Score
Truck-arrow	Bank (cartoon)	
Insect-acorn	Reptile (clown)	
Reptile-clown	Star (ladder)	
Bank-cartoon	Rose (bag)	
Star-ladder	Elephant (glass)	
Raccoon-paper	Truck (arrow)	
Rose-bag	Insect (acorn)	
Elephant-glass	Raccoon (paper)	
	Total score (0-8)	
List B	Recall B	Score
Star-ladder	Elephant (glass)	
Elephant-glass	Insect (acorn)	
Insect-acorn	Reptile (clown)	
Truck-arrow	Rose (bag)	
Reptile-clown	Star (ladder)	
Bank-cartoon	Raccoon (paper)	
Raccoon-paper	Bank (cartoon)	
Rose-bag	Truck (arrow)	
	Total score (0-8)	

List C	Recall C	Score
Rose-bag	Insect (acorn)	
Raccoon-paper	Star (ladder)	
Star-ladder	Truck (arrow)	
Reptile-clown	Rose (bag)	
Elephant-glass	Elephant (glass)	
Insect-acorn	Reptile (clown)	
Bank-cartoon	Bank (cartoon)	
Truck-arrow	Raccoon (paper)	
	Total score (0-8)	Score
List D	Recall D	
Raccoon-paper	Star (ladder)	
Truck-arrow	Rose (bag)	
Star-ladder	Insect (acorn)	
Insect-acorn	Raccoon (paper)	
Rose-bag	Elephant (glass)	
Reptile-clown	Bank (cartoon)	
Bank-cartoon	Reptile (clown)	
Elephant-glass	Truck (arrow)	
	Total score (0-8)	

End of testing: Press red button again (AMS)

3. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

During BP: 4. Saliva sample, time : \_\_\_\_\_

Give task impact questionnaire (1) MEMORY 1

**Matrix Reasoning: *Read instructions***

*After introduction: Press red button (AMS) and begin testing*

**Results: After 3 wrong answers in row stop testing**

Item	Response	Item	Response	Item	Response
A.	2	8.	1	18.	5
B.	5	9.	2	19.	3
C.	4	10.	4	20.	4
1.	3	11.	5	21.	3
2.	3	12.	1	22.	3
3.	2	13.	4	23.	2
4.	2	14.	3	24.	1
5.	3	15.	2	25.	2
6.	1	16.	2	26.	5
7.	5	17.	1		

End of testing: *Press red button*

4. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

During BP: 5. Saliva sample, time : \_\_\_\_\_

Give task impact questionnaire (2) MATRIX

**Verbal Paired Associates 2: *Read instructions; Recall***

*Press red button (AMS) and begin testing*

*Fill in results*

	Recall A	Score
	Bank (cartoon)	
	Reptile (clown)	
	Star (ladder)	
	Rose (bag)	
	Elephant (glass)	
	Truck (arrow)	
	Insect (acorn)	
	Raccoon (paper)	
	Total score (0-8)	

End of testing: *Press red button again (AMS)*

**Read instructions: Recognition**

*Press red button (AMS) begin testing*



*Fill in results*

Item	Circle Y or N		Score
1. rose-bag	Y	N	
2. queen-thumb	Y	N	
3. elephant-glass	Y	N	
4. baseball-forest	Y	N	
5. star-ladder	Y	N	
6. raccoon-paper	Y	N	
7. dish-corner	Y	N	
8. perfume-monkey	Y	N	
9. truck-arrow	Y	N	
10. dance-rocket	Y	N	
11. peanut-pencil	Y	N	
12. bank-cartoon	Y	N	
13. insect-acorn	Y	N	
14. pocket-ribbon	Y	N	
15. candy-typewriter	Y	N	
16. reptile-clown	Y	N	
17. wrinkle-termite	Y	N	
18. rose-bag	Y	N	
19. chicken-submarine	Y	N	
20. star-ladder	Y	N	
21. rain-circus	Y	N	
22. bread-island	Y	N	
23. elephant-glass	Y	N	
24. insect-acorn	Y	N	
	Total score (0-24)		

End of testing: press red button again (AMS)

6. Saliva sample, time: \_\_\_\_\_

Give task impact questionnaire (3) MEMORY 2

### **E) Physical Functioning**

Use 2. stopwatch

*Press red button (AMS)*

*Let participant lie down for 5 min and then measure BP while participant is lying*

5. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

*Let participant stand up and measure BP again*

6. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

*Press red button again (AMS)*

7. Saliva sample, time \_\_\_\_\_

Give questionnaire **REST C**

**F) Recovery and Questionnaire Booklet II**

*Give booklet II to participant, use 2. stopwatch and press red button (AMS)*

*This should take at least 5 min of quietly filling in the questionnaire*

*After 5 min press red button again (AMS)*

7. Casual BP: SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

8. Saliva sample, time \_\_\_\_\_

Give questionnaire **REST D**

**Care giving questionnaire**

**Life events interview**

*Give saliva samples and explain the diary*

*Important: Not during the weekend!*

**COMMENTS ABOUT SESSION** (Note any thing that went wrong, including AMS button pressed out of place):

**Appendix XIII: Diary to Accompany Saliva Sampling****AGEING AND HEALTH STUDY****Participant No.:**\_\_\_\_**Date:**\_\_\_\_\_**Diary to accompany saliva sampling****SCHEDULE FOR SALIVA SAMPLES**

Please use the schedule below in timing the saliva samples and write down the exact time you took the saliva samples together with the tube number.

Please try to use the tubes in order starting with number 9:

Preferred sample time	Tube number	Time taken
Awakening	1	
10 minutes after waking	2	
20 minutes after waking	3	
30 minutes after waking	4	
60 minutes after waking	5	
10.00-10.30	6	
12.00-12.30	7	
14.00-14.30	8	
16.00-16.30	9	
18.00-18.30	10	
20.00-20.30	11	
22.00-22.30	12	

## General information regarding the saliva samples

1. Before each saliva sample, please allow 15 minutes without food and drink to prevent contamination of the sample. If you have eaten something prior to using the Salivette, please rinse your mouth thoroughly with clear water.
2. Remove the plastic cap and take the cotton swab out of the tube.
3. Put the swab in your mouth and chew gently on it for at least two minutes.
4. After this, insert the cotton swab into the plastic tube without touching it with your hands.

## Using the Salivette s in the morning:

❖ *It is easiest if you put the first Salivette, diary and a pen next to your bed before you go to sleep.*

1. Please use the first Salivette (number 1) directly after awakening, at best still in bed and try not to fall asleep again. After the first Salivette, you can get up and move around.
2. Please use Salivette s numbers 2-5 as indicated in the schedule on the front page. After sample number 5 you can have breakfast and/or brush your teeth.
3. Use the following sample tubes numbers 6-12 at the times indicated on the front page, remembering to avoid food and drink for 15 minutes beforehand. Water is OK.
4. If you would like to go to bed before 10pm, you can leave the last sample and simply cross the last box.

❖ *It may be helpful to remember the sample times by setting the alarm on your watch/mobile phone.*

If you **missed one sample**, please indicate this on the schedule and take the next one as indicated.

After the saliva sample, please fill in the time and answer the questions below.

<b>Time</b>					
<b>Tube number</b>					
<b>Place (please circle)</b>	<b>Home</b>	<b>Elsewhere</b>	<b>Home</b>	<b>Elsewhere</b>	<b>Home</b> <b>Elsewhere</b>
<b>Body Position</b>	<b>Lying</b>	<b>Standing</b>	<b>Lying</b>	<b>Standing</b>	<b>Lying</b> <b>Standing</b>
	<b>Sitting</b>	<b>Walking</b>	<b>Sitting</b>	<b>Walking</b>	<b>Sitting</b> <b>Walking</b>
In the last 30 minutes I felt:	See Rating Scale below				
<b>Tired</b>		1   2   3   4   5			1   2   3   4   5
<b>Happy</b>		1   2   3   4   5			1   2   3   4   5
<b>Frustrated or angry</b>		1   2   3   4   5			1   2   3   4   5
<b>I was physically active</b>		<b>Yes</b>		<b>No</b>	<b>Yes</b> <b>No</b>
		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
<b>Stressed</b>		1   2   3   4   5			1   2   3   4   5
<b>Did you talk with others?</b>		<b>Yes</b>		<b>No</b>	<b>Yes</b> <b>No</b>
		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
<b>How pleasant was the interaction?</b>		1   2   3   4   5			1   2   3   4   5
In the last 60 minutes have you...					
<b>Drunk tea/coffee/cola</b>		<b>Yes</b>		<b>No</b>	<b>Yes</b> <b>No</b>
		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
<b>Drunk alcohol</b>		<b>Yes</b>		<b>No</b>	<b>Yes</b> <b>No</b>
		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>

Ratings:

Not at all

Very much

1

2

3

4

5

After the saliva sample, please fill in the time and answer the questions below.

<b>Time</b>			
<b>Tube number</b>			
<b>Place</b> (please circle)	<b>Home</b> <b>Elsewhere</b>	<b>Home</b> <b>Elsewhere</b>	<b>Home</b> <b>Elsewhere</b>
<b>Body Position</b>	<b>Lying</b> <b>Standing</b> <b>Sitting</b> <b>Walking</b>	<b>Lying</b> <b>Standing</b> <b>Sitting</b> <b>Walking</b>	<b>Lying</b> <b>Standing</b> <b>Sitting</b> <b>Walking</b>
In the last 30 minutes I felt:	See Rating Scale below		
<b>Tired</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>Happy</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>Frustrated or angry</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>I was physically active</b>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>
<b>Stressed</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>Did you talk with others?</b>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>
<b>How pleasant was the interaction?</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
In the last 60 minutes have you...			
<b>Drunk tea/coffee/cola</b>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>
<b>Drunk alcohol</b>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>	Yes                  No <input type="checkbox"/> <input type="checkbox"/>

Ratings:

Not at all  
1

2

3

4

Very much  
5

After the saliva sample, please fill in the time and answer the questions below.

<b>Time</b>			
<b>Tube number</b>			
<b>Place</b> (please circle)	<b>Home      Elsewhere</b>	<b>Home      Elsewhere</b>	<b>Home      Elsewhere</b>
<b>Body Position</b>	<b>Lying      Standing</b> <b>Sitting      Walking</b>	<b>Lying      Standing</b> <b>Sitting      Walking</b>	<b>Lying      Standing</b> <b>Sitting      Walking</b>
In the last 30 minutes I felt:	<b>See Rating Scale below</b>		
<b>Tired</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>
<b>Happy</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>
<b>Frustrated or angry</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>
<b>I was physically active</b>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>
<b>Stressed</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>
<b>Did you talk with others?</b>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>
<b>How pleasant was the interaction?</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>	<b>1   2   3   4   5</b>
In the last 60 minutes have you...			
<b>Drunk tea/coffee/cola</b>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>
<b>Drunk alcohol</b>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>	<b>Yes                  No</b>  <input type="checkbox"/> <input type="checkbox"/>

Ratings:

Not at all

Very much

1

2

3

4

5

After the saliva sample, please fill in the time and answer the questions below.

<b>Time</b>			
<b>Tube number</b>			
<b>Place</b> (please circle)	<b>Home</b> <b>Elsewhere</b>	<b>Home</b> <b>Elsewhere</b>	<b>Home</b> <b>Elsewhere</b>
<b>Body Position</b>	<b>Lying</b> <b>Standing</b> <b>Sitting</b> <b>Walking</b>	<b>Lying</b> <b>Standing</b> <b>Sitting</b> <b>Walking</b>	<b>Lying</b> <b>Standing</b> <b>Sitting</b> <b>Walking</b>
In the last 30 minutes I felt:	See Rating Scale below		
<b>Tired</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>Happy</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>Frustrated or angry</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>I was physically active</b>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>
<b>Stressed</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
<b>Did you talk with others?</b>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>
<b>How pleasant was the interaction?</b>	1   2   3   4   5	1   2   3   4   5	1   2   3   4   5
In the last 60 minutes have you...			
<b>Drunk tea/coffee/cola</b>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>
<b>Drunk alcohol</b>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>	<b>Yes</b> <b>No</b> <input type="checkbox"/> <input type="checkbox"/>

Ratings:

Not at all  
1

2

3

4

Very much  
5



**Could you please answer the following questions and return this form with the saliva samples. Many thanks!**

Did anything unusual happen during the day?	Yes	No
If Yes, please provide details		

.....  
 .....

Did you sleep well?	Yes	No
If No, what was the reason?		

.....  
 .....  
 .....

Did you drink alcohol yesterday?	Yes	No
If Yes, what did you drink?		

.....  
 .....

**At the end of the day, please store all samples in the fridge and return them to us as soon as possible. Thank you for taking part in the study.**

**If you have any questions, please contact us:**

<b>Sabine Kunz</b>	<b>0207 679 5634</b>
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<b>Caroline Wright</b>	<b>0207 679 1702</b>
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<b>Lindsey Emerson / Liz Cort</b>	<b>0207 679 5973</b>
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<b>Voicemail</b>	<b>0207 679 1804</b>
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**Thank you very much for taking part in this study!**

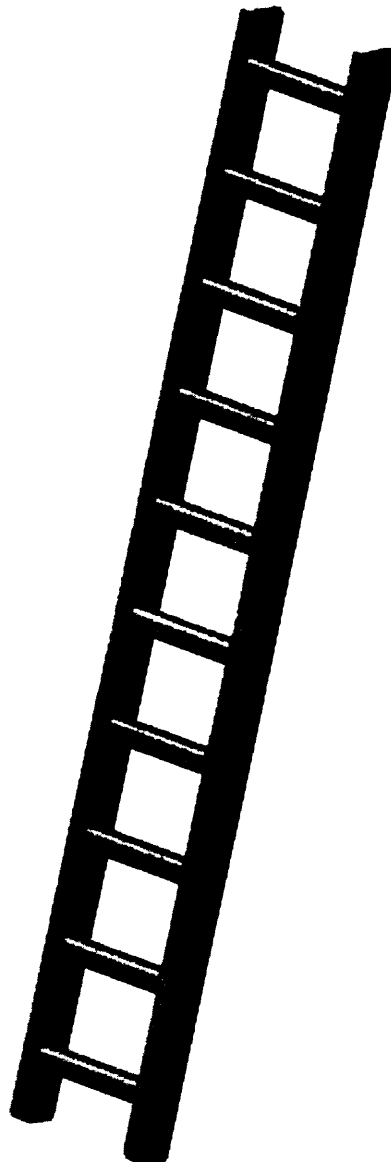
## **Appendix XIV: Ladder Measure of Subjective Social Status**

**Think of this ladder as representing where people stand in our society.**

At the **top** of the ladder are the people who are the best off those who have the most money, most education and best jobs. At the **bottom** are the people who are the worst off who have the least money, least education, and the worst jobs or no job. The higher up you are on this ladder, the closer you are to the people at the very top and lower you are, the closer you are to the people at the very bottom.

**Where would you place yourself on this ladder?**

**Please place a large "X" on the rung where you think you stand.**



## Appendix XV: Objective Measures of Social Status

### EDUCATION AND WORK HISTORY

1. What was your main occupation for most of your working life?  
\_\_\_\_\_
2. At what age did you leave school? \_\_\_\_\_ years old
3. What is the highest qualification you have attained, either while at school or after you left school?

	Please circle the number below that most applies to you
No qualification	1
<ul style="list-style-type: none"> <li>• CSE Grades 2-5</li> <li>• GCSE Grades D-G</li> </ul>	2
<ul style="list-style-type: none"> <li>• School Certificate</li> <li>• CSE Grade 1</li> <li>• GCE 'O' Level</li> <li>• GCSE Grades A-C</li> <li>• Scottish SCE/SUPE Ordinary</li> <li>• Scottish School Leaving Certificate (SLC) Lower</li> <li>• City and Guilds Craft/Intermediate/Ordinary/Part 1</li> </ul>	3
<ul style="list-style-type: none"> <li>• Higher School Certificate</li> <li>• Matriculation</li> <li>• GCE 'A' Level / 'S' Level</li> <li>• Scottish SCE/SLC/SUPE Higher</li> </ul>	4
Apprenticeship	5

Professional Qualification For example <ul style="list-style-type: none"> <li>• Teachers training qualification</li> <li>• Nursing qualification</li> </ul>	7
Degree, including higher degree	8
Other _____ (please explain)	

## FINANCIAL QUESTIONS

1. During the last year have there been any major things that you or your family really needed to buy but have not been able to afford? (please circle your response)

**NO**

**YES**

2. All things considered, how satisfied or dissatisfied are you with your present financial situation? (please circle your response)

<b>Completely dissatisfied</b>				<b>Completely satisfied</b>
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>

3. How would you rate your present financial situation? (please circle your response)

<b>Best imaginable</b>				<b>Worst imaginable</b>
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>

## Appendix XVI: CES-D and LOT Measures of Depression and Optimism

### QUESTIONS ABOUT YOUR FEELINGS

Below is a list of ways that you might have felt or behaved recently. Please tell us how often you have felt this way during the **past week**.

1. I was bothered by things which don't usually bother me.

1	2	3	4
Rarely or none of the time	Some or a little of the time	a moderate amount of time	most or all of the time

2. I did not feel like eating; my appetite was poor

1	2	3	4
Rarely or none of the time	Some or a little of the time	a moderate amount of time	most or all of the time

3. I felt that I could not shake off the blues even with the help of my family or friends.

1	2	3	4
Rarely or none of the time	Some or a little of the time	a moderate amount of time	most or all of the time

4. I felt that I was just as good as other people.

1	2	3	4
Rarely or none of the time	Some or a little of the time	a moderate amount of time	most or all of the time

5. I had trouble keeping my mind on what I was doing.

1	2	3	4
Rarely or none of the time	Some or a little of the time	a moderate amount of time	most or all of the time

6. I felt depressed.

1	2	3	4
Rarely or none of the time	Some or a little of the time	a moderate amount of time	most or all of the time

7. I felt that everything I did was an effort.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

8. I felt hopeful about the future.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

9. I thought my life had been a failure

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

10. I felt fearful.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

11. My sleep was restless.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

12. I was happy.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

13. I talked less than usual.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

14. I felt lonely.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

15. People were unfriendly.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

16. I enjoyed life.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

17. I had crying spells.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

18. I felt sad.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

19. I felt that people dislike me.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--

20. I could not get going.

<b>1</b> <b>Rarely or none of the time</b>	<b>2</b> <b>Some or a little of the time</b>	<b>3</b> <b>a moderate amount of time</b>	<b>4</b> <b>most or all of the time</b>
---	---	--	--



## QUESTIONS ABOUT YOU

Using the scale below please write the appropriate letter in the box beside each statement which is true for you personally.

<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>
<b>I agree a lot</b>	<b>I agree a little</b>	<b>I neither agree or disagree</b>	<b>I disagree a little</b>	<b>I disagree a lot</b>

a. In uncertain times I usually expect the best.	
b. It's easy for me to relax.	
c. If something can go wrong for me it will.	
d. I always look on the bright side.	
e. I'm always optimistic about my future	
f. I enjoy my friends a lot.	
g. It's important for me to keep busy	
h. I hardly ever expect things to go my way.	
i. Things never work out the way I want them to.	
j. I don't get upset easily.	
k. I'm a believer in the idea that 'every cloud has a silver lining'.	
l. I rarely count on good things happening to me.	

## **Appendix XVII: Information Sheet (Study 3)**

**Royal Free and University College Medical School**  
**UNIVERSITY COLLEGE LONDON**



**DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH**

# **FAMILY HISTORY AND HEALTH STUDY**

## **Information Sheet**

**Principal investigators:** Professor Andrew Steptoe and Professor Jane Wardle

**Investigating team:** Caroline Wright, Bev Murray, and Katie O'Donnell

### ***What is this study about?***

This study is concerned with the way in which we react biologically to mental stress, and the role these responses may play toward risk of future serious diseases. It is funded by the Medical Research Council, Cancer Research UK, and the British Heart Foundation. It is part of a series of studies into how our everyday life, family history and experiences can stimulate biological responses that may, in the long term, contribute to the development of cardiovascular and other diseases. This important area of medical research will contribute to the understanding of how and why serious physical illness may develop, and will help us devise better methods of prevention.

### ***Who can take part? (Our 'Participant Profile')***

- We are looking for healthy male and female volunteers aged between 18 and 25 years.
- Participants should be in good health and not taking any medication (including antidepressants). Although taking oral contraception is ok.
- Due to the nature of one of the tasks participants should not be colour blind
- On the day of testing, participants will need to be without any cold or flu symptoms, and should avoid taking aspirin, ibuprofen or antibiotics for up to 10 days before the session (paracetamol can be used instead).
- Caffeine should also be avoided for 12 hours prior to, and during the study.
- Alcohol should also be avoided for the 12 hours before testing.

- We will need to be able to contact (by post or email) at least one blood-related parent in order for them to fill in a simple questionnaire on their current health and risk factors for disease. This information is needed for us to be able to interpret the profile of biological responses obtained and to gain full details of ones health background.
- Participants will also be supplied with a standard meal (usually pasta salad or similar) so must not have any particular dietary requirements (all meals are vegetarian).

### ***What will I have to do?***

The study involves you spending an afternoon (from 1.00-4.30) with us at the Department of Epidemiology and Public Health, University College London, situated on Torrington Place (Gower Street campus). When you agree to take part in the study, we will arrange a convenient date for you to attend a session, which will begin at 12.30 p.m. and will take approximately three and a half hours. If you happen to have a cold or have had to take medicine shortly before, please get in touch beforehand so that we can reschedule the appointment.

Be sure to wear comfortable clothing. When you arrive at the department, one of our team members will meet you at the reception desk and take you to the rooms where the study takes place. Before starting, we will ask you to fill in a consent form, and make sure that any questions you may have are answered.

### ***What happens during the study?***

- First, we will give you a light meal consisting of pasta, salad and fruit. It is crucial that you don't eat lunch before hand, although a light breakfast is allowed.
- Second, our research nurse will take some physical measures, namely height, weight, waist and hip circumference, and body composition. The latter measure requires removal of shoes and socks. At this point you will be asked to provide a saliva sample (for the measurement of the stress hormone cortisol).
- Next, we will need to fit you up to a portable heart monitor. This is completely painless and involves us fitting some small electrode-pads to your skin
- After the physical measurements, you will be seated in a comfortable chair and a needle will be inserted in a vein in your lower arm or the back of your hand. Two small cuffs will be attached to two of your fingers. A blood pressure cuff will be attached to your arm at certain times, so it would be helpful if you wear clothing with loose sleeves. In the 30 minutes following this, there will be a rest period during which you may read or watch one of our videos. Please bring something along to read if you want (provided it is fairly relaxing!). You will then be asked to perform two tasks. One is a visual problem solving task, and the second is a speech task. These tasks do not require any special skills. Blood will be taken at various times during the study and you will be asked about your mood during the procedure. After

the task you can relax again, this time for 90 minutes, during which time you will complete a questionnaire and then read or watch videotapes.

- At the end of the study you will also be given a set of tubes for collecting 6 saliva samples at home. We will show you how to take the samples, and when to take them. You will be given a short “diary” to fill in with each saliva sample, recording your activity, mood etc. We will arrange for you to drop these off in the Department when you have done them.
- After the experiment we will be happy to discuss all that happened during the session and answer any additional questions you may have.

***During the session, what measurements do we want to take from you and how will it be done?***

➤ *Measurements of blood pressure*

During the session, two small cuffs placed around the fingers of one hand will measure your blood pressure. You will feel the cuffs pulsating slightly, but you'll soon get used to this sensation and it will not be uncomfortable. We will also take blood pressure measurements now and again with a cuff placed around the arm.

➤ *Saliva measurements*

You will be asked to provide eight saliva samples throughout the session and a further six to complete at home. This involves placing a small cotton swab under the tongue for 2 minutes. The saliva samples will allow assessment of hormone activity.

➤ *Blood measurements*

In order to examine physiological function, we will need to take some blood from you during the session. This is why we insert a needle in the back of your hand or lower arm. This needle remains comfortably in place throughout the session. The insertion of the needle may cause slight discomfort at first, which is why we give you a 30-minute rest afterwards to get used to the needle. We draw blood samples from the inserted needle five times throughout the session. This is a painless procedure, and is designed to cause minimum discomfort. The measurements we obtain from your blood are indicators of physiological activity, and will include cortisol, leptin, and cytokines.

➤ *Cardiovascular measurements*

In order to examine cardiovascular function you will also be fitted with a heart monitor for the duration of the study session. This will be a pain free procedure and will involve having electrodes placed on your chest and back – similar to an ECG (electrocardiogram) – in order for us to look at factors including heart rate.

***What are the benefits?***

We will give you information about your blood pressure, heart rate and body-fat composition after the session. Importantly, you will be helping us understand more about the way everyday challenges may be linked with family history and the risk of

physical illness in the future. This research may go on to help you and others in the long-term. You will also receive an honorarium of £30 as a token of our gratitude once you have completed and returned the saliva samples that are to be taken at home on a separate day (usually the following day).

***What if I change my mind during the study?***

If at any point and for any reason you do not want to carry on, then you may stop. There are no consequences of withdrawal from the study.

***What happens to the information?***

All the information that we get from this study about you, including your name, will be confidential, and will only be used for medical research purposes. Data from all volunteers will be combined and it will not be possible to identify individuals within published results.

***What happens at the end of the study?***

At the end of the study, we shall send you a brief summary of our findings.

***Do I have to sign anything?***

We will ask you to sign a Consent Form. This is to show that you understand what is involved and that you have read this Information Sheet. You can still withdraw from the study at any time.

***What if I have more questions or do not understand something?***

If you have any queries, or would like more information about the study, please feel free to telephone the research team at 020 7679 5698. Any member of our team will be happy to answer your questions. Since we cannot always be in our office, you might get an answer machine, but if you leave your name and telephone number, a member of our team will get back to you as soon as possible.

January 2004

## Appendix XVIII: Protocol (Study 3)

### Family History and Psychobiological Response to Mental Stress Study

#### STUDY PROTOCOL

Participant ID			
Date		Date of Birth	
Start time		Age	
Time of awakening		Gender	

**\* INITIALISE AMS\* - Set time on computer**

#### ***Cycling Lab (12:30h):***

- Lunch
- Information sheet and consent form
- Confirm health status detailed in information sheet
- Check instructions were followed: medication / alcohol / exercise / caffeine
- Time and content of last meal  
.....
- How did they travel to the session? (did anything unusual happen?)  
.....
- Brief verbal explanation of study
- First **SALIVA (1)** sample (2 min under tongue, chewing gently) STOP WATCH 2 MIN

Physical Measures			
Height	M	Weight	kg
Hip measure	Cm	Waist measure	cm

- Body Stat Measure
- Skin fold measure 1) Biceps .....cm 2) Triceps .....cm 3) Subscapular .....cm  
4) Suprailiac .....cm
- Smoker? No Yes Time of last cigarette..... CO level .....
- Attach AMS monitor electrodes Measure electrode distance Front.....cm  
Back.....cm
- check complexes and resistance

***Stress Lab (14:00h):*** (check room temperature is 22°)

- Offer opportunity to use toilet on the way to stress lab before needle insertion
- **(Initialise Portapres: enter physical data and set cuffs to 1-min switching interval)**
- Needle insertion and heat pad
- Attach Portapres cuffs (middle and ring) and height line (explain camera and uncrossed legs)
- Switch Portapres (PP) on (START/STOP button) to ensure both cuffs work OK
- Casual BP SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_ (habituation)
- PP off (START/STOP button), **change to 30-min cuff interval** (press *finger* button, arrow down to select 30 min switching, press START/STOP to confirm, store settings: arrow right to select yes, press START/STOP to confirm) and leave in ready mode

**30min Rest Period (Start 14:10h)**

**START CLOCK**

- PRESS AMS EVENT BUTTON

TIME NOW:

- **At 10 min:** PP on (START/STOP) (remember to monitor times of cuff switch)

**ENSURE PP START/STOP BUTTON NOT PRESSED DURING  
THE SESSION FROM NOW ON**

**ENSURE DISPLAY READS BETWEEN 30/06 AND 30/28 BEFORE RECORDING  
EVENTS**

- **At 25 min:** (check display at 30/06-30/28)
- © **Calibration off** © press **PP event** button
- **At 30 min:**
- © Press **PP & AMS event** button © **calibration on**
- © Rest Questionnaire
- © Casual BP    SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_
- \*wait 1 minute, sitting quietly\*
- © Casual BP    SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_
- © **SALIVA (2)** STOP WATCH 2 MIN and
- © **BLOOD (1)** Start time ..... Stop time .....

## Stress Tasks (14:40h)

TIME NOW:

**STROOP**

Give written Stroop instructions. *While they are reading instructions set up Stroop.*

- Talk the participant through the practice
- Let the participant have a go at the practice until they have the idea (within reason).
- *(check display at 30/06-30/28)*
- **Calibration off** © **START STROOP** (F3 return) © press PP & AMS **event** button
- At end of task press 3 enter, 3 enter on computer to exit, then press PP & AMS **event** button © **calibration on**
- Task impact questionnaire (1) and while doing this **SALIVA (3) STOP WATCH 2 MIN**

**SPEECH**

Check time before switching on portapres (*display should read between 30/06 and 30/28 for the event, plus 4-5 min explanation time = 30/11-30/28*)

- Explain the task to the participant. Then hand them the written instructions and say they will have 2 min to prepare.
- **Calibration off © START TASK** (=2 min preparation time and 3 min speech) © press PP & AMS **event** button



- **Start stop watch and time 2 min preparation time (outside room). Then return and instruct to start speech. Time for 3 min.**
- **At end of 3 min speech say you can stop now, press PP & AMS event button © calibration on**
- **Task impact questionnaire (2)**

Recovery period (15:00 – 16:30h)

RE-START CLOCK

Immediately post-task:

TIME NOW:
-----------

- **PRESS AMS EVENT BUTTON**
- **Casual BP    SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_**
- **SALIVA (4) STOP WATCH 2 MIN and**
- **BLOOD (2) Start time ..... Stop time .....**
  
- **Check calibration is on**
  
- **At 10 min: (check display at 30/06-30/28)**  
 © **Calibration off** © **press PP event button**
- **At 15 min:**  
 © **Press PP event button** © **calibration on**  
 © **Recovery (1) Questionnaire**  
**SALIVA (5) STOP WATCH 2 MIN**
  
- **At 25 min: (check display at 30/06-30/28)**  
 © **Calibration off** © **press PP event button**
- **At 30 min:**  
 © **Press PP event button** © **calibration on**  
 © **Recovery (2) Questionnaire**  
**SALIVA (6) STOP WATCH 2 MIN**
  
- **At 40 min: (for 5 min) (check display at 30/06-30/28)**

© **Calibration off** © press PP **event** button

➤ **At 45 min:**

© Press PP **event** button

© **Calibration on**

© Recovery Questionnaire (3)

© **SALIVA (7)** STOP WATCH 2 MIN and

© **BLOOD (3)** Start time ..... Stop time .....

(should finish by 51 min)

**\*\*COMPLETE QUESTIONNAIRE\*\***

➤ **At 1h 25m:** (for 5 mins) (*check display at 30/06-30/28*)

© **Calibration off** © press PP **event** button

➤ **At 1h 30m:**

➤ © Press PP & AMS **event** button © **Calibration on**

© **Turn Portapres off (START/STOP button)**

➤ Casual BP    SBP \_\_\_\_\_ DBP \_\_\_\_\_ HR \_\_\_\_\_

➤ Recovery Questionnaire (4)

➤ **SALIVA (8)** STOP WATCH 2 MIN and

**BLOOD (4)** Start time ..... Stop time .....

➤ Needle out

➤ Take out Portapres air hose and cable, remove cuffs and height line and turn off at wall.

➤ Remove AMS electrodes

## **Appendix XIX: Stress Task 1 Instructions**

### **STROOP TASK INSTRUCTIONS**

In this task you will be presented with one word and asked to name the colour in which the word is printed.

#### **RED**

The answer you need to give here is **BLUE**, as that is the colour the word is printed in. The answer is **NOT RED**, that is the colour you read.

To give the correct answer you need to choose from four possibilities.

**BLUE**

**GREEN**

**YELLOW**

As the answer is **BLUE**, you need to select the word **BLUE** (although it is written as green). So to give the answer you need to choose the word **BLUE**, not the word that is written in blue. If you do not answer quickly enough the computer will give you the correct answer and move on to the next trial.

We will start with a short practice test now, so that you can get more familiar with the procedure before we start the real task.

Once we start the real task, you will perform the task for 5 minutes and the number of correct responses will be recorded.

## **Appendix XX: Stress Task 2 Speech Scenario**

Participant code:

Session no:

### **Role-Play Speech Scenario 1 – “Pickpocket”**

Every day, we find ourselves having to deal with many difficult social situations. Imagine that you are in a busy department store and that you are trying to squeeze past a group of shoppers. You notice a purse on the floor. You bend down and pick it up and then open the purse to see if there is any identification inside. Suddenly, you feel a hand grab you on the shoulder and a stern voice says “Ok, come with me, I saw you steal that purse”. As you are being led to the manager’s office you realise that the security guard believes you are a pickpocket. The police are called immediately, and it is up to you to defend yourself.

What we want you to do in this task is to speak as if you were trying to defend yourself to the police. Remember that if you do not give a clear account of what happened, the police are likely to prosecute you. If the case goes any further, you may be answering questions before a judge. Try to conjure up the feelings you would experience in this situation, and think quickly about how best to defend yourself.

Here are some things you might do. Firstly, describe what actually happened and try to give reasons to the police why you wouldn’t have stolen the purse. Secondly, think about how you would feel about the security guard for making this serious mistake about you. Perhaps you would criticise the department store for employing incompetent and trigger-happy guards. Thirdly, think of ways that you could convince the police that you are not the sort of person who would commit this crime.

## **Appendix XXI: Stress Task 2 Instructions**

### **ROLE-PLAY SPEECH INSTRUCTIONS**

In this task, I will present you with a hypothetical situation involving a real-life problem that could happen to anyone. In just a moment, I will read a description of this situation to you and then you will be given 2 minutes in which to prepare a response or story around the situation. You may look over what I'll read to you during your preparation time. Then, at the end of your preparation time, I will ask you to give your story for 3 minutes, talking at the video camera in front of you.

Try to imagine the emotions and feelings that you would experience if you were confronted by the situation that will be described. It's very important for our research that you not only try your hardest but that you also speak for the entire time. In addition, your task will be tape-recorded and later replayed by 3 of our laboratory staff and judged for "fluency, plausibility and confidence".

O.K. Here's your situation. (Read scenario to subject).

---

O.K. ANY QUESTIONS??

Fine. During your role-play speech, the measurements on your blood vessels will continue, so please keep as still as possible. Just try to conjure up the reactions you would experience in this stressful situation, and some heartfelt responses. You will now have two minutes to imagine yourself in this situation before you are asked to begin speaking. You may make notes if you wish. Once you have begun your speech, please try to continue without a break until you are told that the 3-minute period has come to an end. If necessary you can repeat things that you have already said.

Here's the written description of your situation – please make any notes on it if you wish to. I'll be back at the end of your 2-minute preparation time.

## **Appendix XXII: Study 3 Parental Letter and Family History Questionnaire**

**Royal Free and University College Medical School  
UNIVERSITY COLLEGE LONDON  
PSYCHOBIOLOGY GROUP  
DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH**

Gower Street Campus  
1-19 Torrington Place  
London WC1E 6BT



Dear

### **Biological responses to stress in young adults**

Your daughter has recently taken part in a research study into the influence of mental stress on biological responses. This study, which is funded jointly by the Medical Research Council, the British Heart Foundation, and Cancer Research UK, will help us understand how social and psychological factors “get under the skin”, and contribute to the development of serious illnesses.

As part of this study, we need to collect information about the health of the parents of the students who have participated. This is because we know that family history of cardiovascular disease influences the biological measures we have obtained from your son/daughter, and we need to take these factors into account. I am therefore writing to ask whether you can provide this information. Attached to this letter is a short questionnaire, and I should be most grateful if you could complete it and return it to me. The information you provide will be completely confidential, and will be used for research purposes only. The information will not be divulged to anyone else, and you will not be identified personally in any of the scientific work we do. It is, however, very important for our research that we obtain as much information as possible about the cardiovascular history of both the mothers and fathers of participants in this study.

Once you have completed the questionnaire, please return it in the attached Freepost envelope. It is not necessary to stamp the envelope. Thank you very much for your help. If you would like more information or to talk to someone about the study, please contact either Caroline Wright

Bev Murray

Yours sincerely

**Andrew Steptoe,  
British Heart Foundation Professor of Psychology**

Name of student .....

Are you the Student's MOTHER / FATHER (please delete as appropriate)

Date .....

Please provide as much information as you can:

1. Has a doctor ever told you that you have **heart disease**? Yes / No  
If 'yes', when were you diagnosed? .....
2. Has a doctor ever told you that you have **high blood pressure**? Yes / No  
If 'yes', when were you diagnosed? .....
3. Has a doctor ever told you that you have **diabetes**? Yes / No  
If 'yes', when were you diagnosed? .....
4. Do you smoke? Yes / No
5. If you are not a smoker now, did you smoke in the past? Yes / No  
If 'yes', for how many years did you smoke in total? .....
6. Has a doctor ever told you that you have **high cholesterol**? Yes / No  
If 'yes', when were you told? .....
7. What is your weight (in either stones/pounds or kilograms)? .....
8. What is your height (feet and inches or centimetres)? .....
9. What is your waist circumference? .....
10. How old are you? .....
11. Can you recall the birth weight of your son / daughter? .....
12. What is your current job or occupation? .....

PTO

13. Are either of your (biological) parents still alive? MOTHER: Yes / No / Don't know  
FATHER: Yes / No / Don't know

14. If not, how old were they when they died? MOTHER:.....  
FATHER:.....

15. Can you recall the cause of death? MOTHER:.....  
FATHER:.....

**For the next section please provide information, where possible, for both your (biological) parents, even those which are no longer alive.**

16. Have either of your parents ever been told that they have *heart disease*? Mother: Yes / No / Don't know  
Father: Yes / No / Don't know  
Mother: .....  
If 'yes', at what age were they diagnosed? Father: .....

17. Have either of your parents ever been told that they have *high blood pressure*? Mother: Yes / No / Don't know  
Father: Yes / No / Don't know  
Mother: .....  
If 'yes', at what age were they diagnosed? Father: .....

18. Have either of your parents ever been told that they have *diabetes*? Mother: Yes / No / Don't know  
Father: Yes / No / Don't know  
Mother: .....  
If 'yes', at what age were they diagnosed? Father: .....

19. Have either of your parents ever been told that they have *High Cholesterol*? Mother: Yes / No / Don't know  
Father: Yes / No / Don't know  
Mother: .....  
If 'yes', at what age were they diagnosed? Father: .....

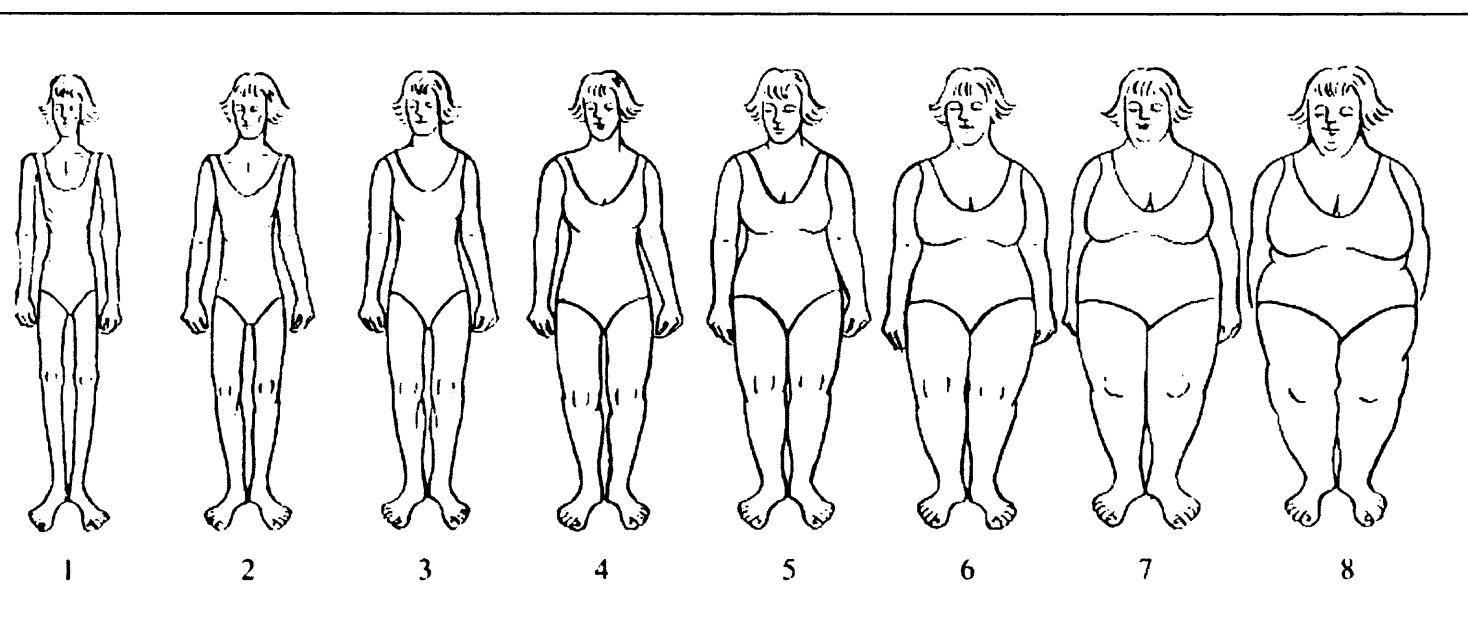
20. Have either of your parents ever smoked? Mother: Yes / No / Don't know  
Father: Yes / No / Don't know  
Mother: .....  
If yes, how many years in total did they smoke for? Father: .....



Next we would like you to look at the set of drawings below. They represent people of different body shapes. We would like you to circle the number below the figure which best describes your body shape *at the moment*. There are no right or wrong answers so please try to be as honest as possible.

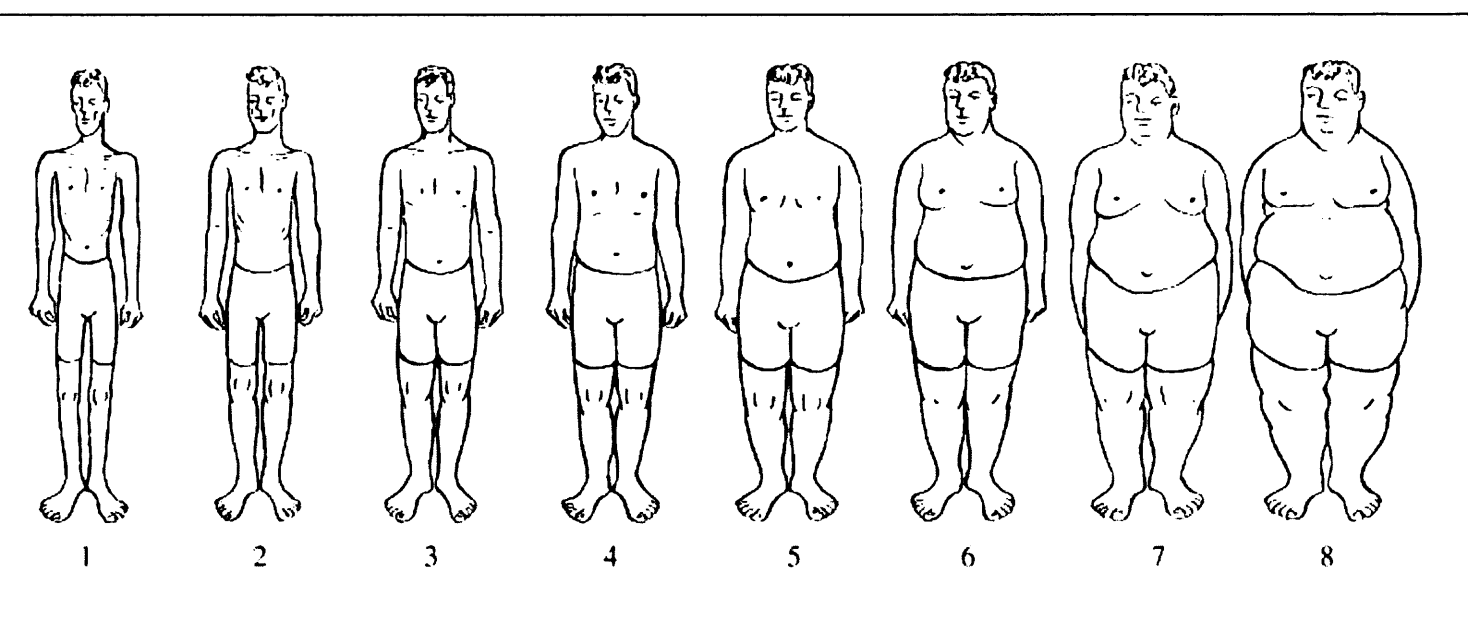
**a) IF YOU ARE FEMALE**

We would like you to tell us which of these figures best represents your *current* body shape.



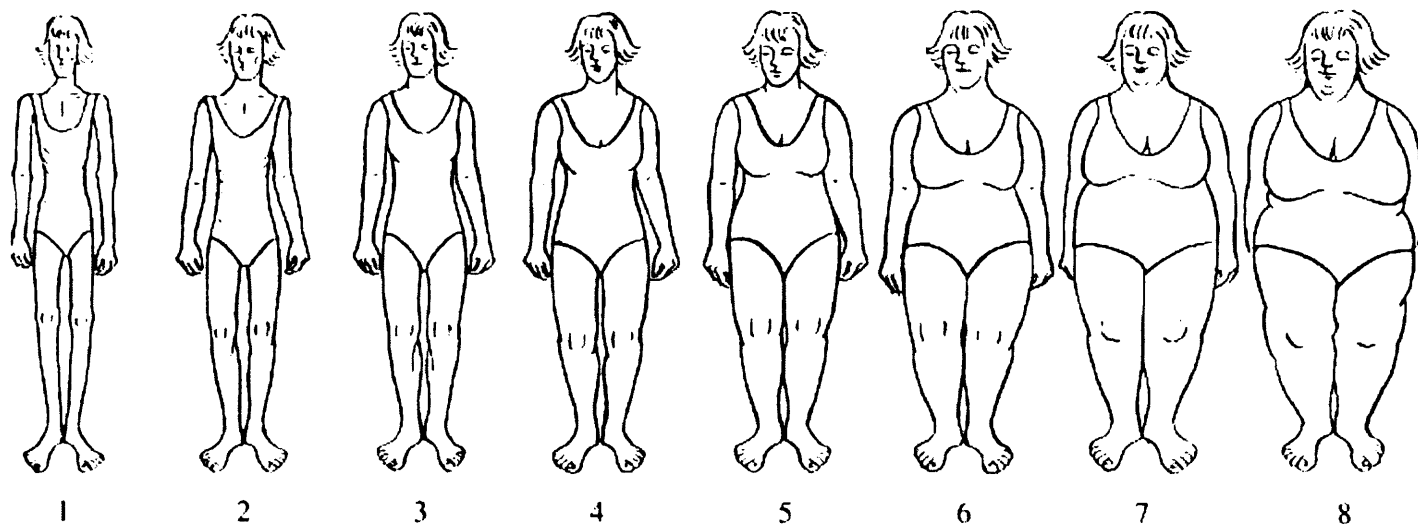
**b) IF YOU ARE MALE**

We would like you to tell us which of these figures best represents your *current* body shape.



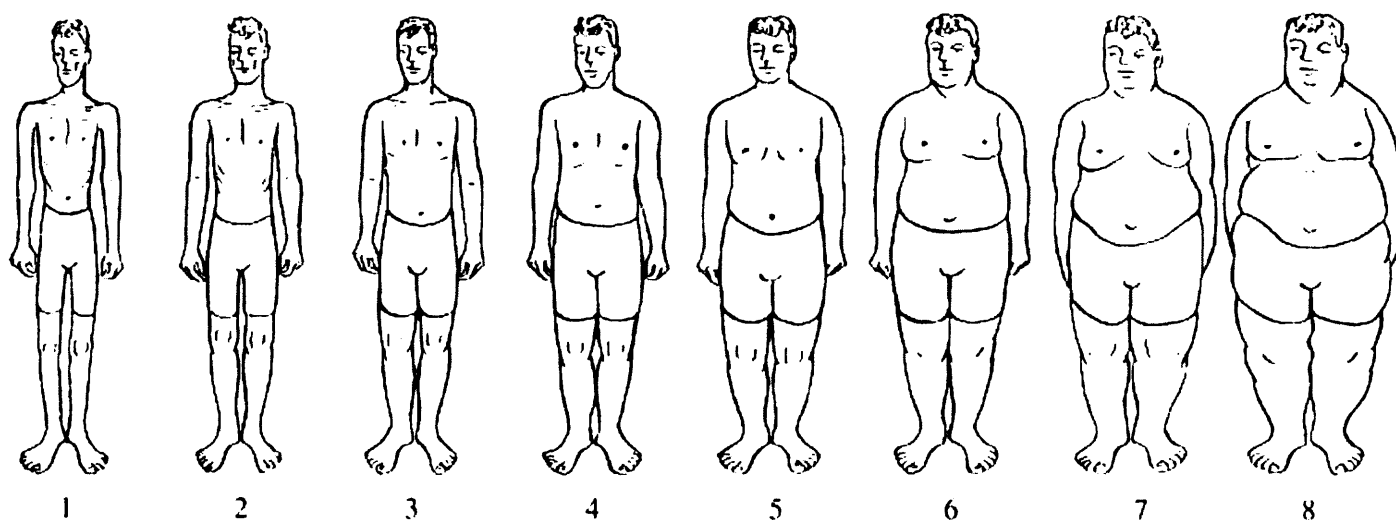
**c) YOUR MOTHER**

We would like you to tell us which figure best represents your mother's body shape.



**d) YOUR FATHER**

We would like you to tell us which figure best represents your father's body shape.



**Thank-you for your help.**

Please return this questionnaire in the envelope provided or to the Family History and Health Study, Psychobiology Group, Dept of Epidemiology and public health, UCL, 1-19 Torrington Place, London, WC1E 6BT

## **Appendix XXIII: Information Sheet (Study 4)**

# **Royal Free and University College Medical School**

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH

Gower Street Campus  
1-19 Torrington Place  
London WC1E 6BT



## **A Study of Mood and the Immune System**

### **INFORMATION SHEET**

Principal investigator: Professor Andrew Steptoe.

Investigating team: Caroline Wright, Dr. Philip Strike, Lindsey Emmerson, Bev Murray and Dr Lena Brydon.

#### **What is this study about?**

This study is funded by the Medical Research Council and the British Heart Foundation, and aims to explore how behaviour and mood influence the inflammatory effects of vaccination.

#### **Who can take part?**

We are looking for male students (postgraduate or undergraduates) aged between 18 & 30 years who are generally fit and well. Volunteers should not be taking any regular medicines and be non-smokers. We would like to include people who do not have a history of significant physical or mental illness. Volunteers should also not recently (within the last 6 months) have received any vaccinations.

#### **What will I have to do?**

The study will take approximately 1 hour of your time over the course of one day and will involve you coming to the Department of Epidemiology and Public Health, University

College London, situated on Torrington Place. When you agree to take part in the study, we will arrange a convenient date for you to attend a session, which will begin at 9:00 a.m. When you arrive at the department, one of our team members will meet you at the reception desk and take you to the rooms where the study takes place. We will initially ask you a few basic health questions and ask you to fill in a consent form, making sure that any questions you may have are answered. If you happen to have a cold or flu or have had to take medicine shortly before, please get in touch beforehand so that we can reschedule the appointment. **You should not have taken antibiotics for at least 10 days prior to your appointment and aspirin or ibuprofen for at least 72 hours before coming to see us.**

### **What happens during the study?**

At the beginning of the study you will be asked to fill in a questionnaire about how you are feeling and about stresses in your daily life. We will take a small 10ml blood sample and you will receive a small painless injection which will either be the typhoid vaccine or placebo. At the time of the study neither you nor we will know whether or not you've had vaccine or placebo, but you will be informed later on. We will then take a saliva sample and give you a brief (5 minute) questionnaire to complete. This initial part of the procedure will take approximately 30 minutes. You will then be free to do whatever you want until we need to see you again at 10.30, 12.00 and 3.00. These sessions should take no longer than 10 minutes each and will involve the taking of your blood pressure, body temperature, saliva samples and the completion of another brief questionnaire (one other blood sample will also be required at the 12.00 session).

### **What are the benefits?**

We will give you information about your blood pressure and heart rate after the session. Importantly, you will be helping us understand more about the way different behaviours affect the body, and this research may help you and other people in the long term. You will receive a gift of £30 as a token of our gratitude.

### **What if I change my mind during the study?**

If at any point and for any reason you do not want to carry on, then you may stop. There are no consequences of withdrawal from the study.

### **What happens to the information?**

All the information that we get from this study about you, including your name, will be confidential, and will only be used for medical research purposes. Your name and personal details will be kept in a separate location from the data files. Data from all volunteers will be combined and it will not be possible to identify individuals within published results.

### **What happens at the end of the study?**

At the end of the study, we shall send you a brief summary of our findings.

**Do I have to sign anything?**

We will ask you to sign a Consent Form. This is to show that you understand what is involved and that you have read this Information Sheet. You can still withdraw from the study at any time.

**What if I have more questions or do not understand something?**

If you have any queries, or would like more information about the study, please feel free to contact the research team by calling 020 7679 1804 or by emailing [c.wright@public-health.ucl.ac.uk](mailto:c.wright@public-health.ucl.ac.uk). Any member of our team will be happy to answer your questions. Since we cannot always be in our office, you might get an answer machine, but if you leave your name and telephone number, a member of our team will get back to you as soon as possible.

## Appendix XXIV: Profile of Mood States

Below is a list of words that describe feelings people have. Please read each one carefully, then circle one number to the right of the word to indicate the answer which best describes the extent to which you have this feeling now.

Participant number.....

Time.....

The numbers refer to these phrases:

Profile number.....

- 0 Not at all
- 1 A little
- 2 Moderately
- 3 Quite a lot
- 4 Extremely

For example Angry 0 1 2 3 4 would indicate that you are currently feeling moderately angry.

		Not at all	A little	Moderately	Quite a lot	Extremely				Not at all	A little	Moderately	Quite a lot	Extremely
(1)	Tense	0	1	2	3	4		(18)	Nauseated	0	1	2	3	4
(2)	Feverish	0	1	2	3	4		(19)	Listless	0	1	2	3	4
(3)	Worn out	0	1	2	3	4		(20)	Nervous	0	1	2	3	4
(4)	Lively	0	1	2	3	4		(21)	Lonely	0	1	2	3	4
(5)	Confused	0	1	2	3	4		(22)	Muddled	0	1	2	3	4
(6)	Shaky	0	1	2	3	4		(23)	Cheerful	0	1	2	3	4
(7)	Aching joints	0	1	2	3	4		(24)	Exhausted	0	1	2	3	4
(8)	Sad	0	1	2	3	4		(25)	Gloomy	0	1	2	3	4
(9)	Active	0	1	2	3	4		(26)	Sluggish	0	1	2	3	4
(10)	On edge	0	1	2	3	4		(27)	Headache	0	1	2	3	4
(11)	Energetic	0	1	2	3	4		(28)	Weary	0	1	2	3	4
(12)	Hopeless	0	1	2	3	4		(29)	Bewildered	0	1	2	3	4
(13)	Relaxed	0	1	2	3	4		(30)	Alert	0	1	2	3	4
(14)	Unhappy	0	1	2	3	4		(31)	Efficient	0	1	2	3	4
(15)	Uneasy	0	1	2	3	4		(32)	Forgetful	0	1	2	3	4
(16)	Can't concentrate	0	1	2	3	4		(33)	Guilty	0	1	2	3	4
(17)	Fatigued	0	1	2	3	4		(34)	Vigorous	0	1	2	3	4



**Appendix XXV: Questionnaire Booklet (Study 4)**

**MOOD AND THE IMMUNE SYSTEM  
QUESTIONNAIRE**

ALL ANSWERS ARE STRICTLY CONFIDENTIAL

Thank you for your help

**PLEASE ANSWER ALL QUESTIONS AND INDICATE THE ANSWERS WHICH  
YOU FEEL ARE MOST APPROPRIATE TO YOU PERSONALLY**

**Participant Number.....**

**Age.....**

## QUESTIONNAIRE

### FINANCE

**Q1.** These items concern the types of difficulty that can arise because of economic problems. Please use the scale to indicate whether each item **is true for you at the present time:**

At the present time	No difficulty	With some difficulty	Very great difficulty
a. Are you able to afford furniture or household equipment that needs to be replaced?	1	2	3
b. Do you have enough money for the kind of food you should have?	1	2	3
c. Do you have problems in paying your bills?	1	2	3
d. Do you have enough money for the kind of clothing you should have?	1	2	3
e. Are you able to afford to replace major items (such as a car) when you need to?	1	2	3
f. Do you have enough money for the leisure activities you want?	1	2	3
g. Are you able to afford suitable accommodation?	1	2	3
h. At the end of the month, do you have:	Some money left over	Just enough to make ends meet	Not enough to make ends meet



## WORK / STUDY

**Q2. The following questions are about your study. For each please indicate the one answer that best describes your work or the way you deal with problems occurring at university.**

	Often	Sometimes	Seldom	Never /Almost never
a. Do you have to work very fast?	1	2	3	4
b. Do you have to work very intensively?	1	2	3	4
c. Do you have enough time to do everything?	1	2	3	4
d. Do different lecturers demand things from you that you think are hard to combine?	1	2	3	4
e. Is your work psychologically demanding?	1	2	3	4

**Q3.** The following questions are about you life as a student. Please indicate if any of these events have happened to you at any time **over the past two weeks**. If they have, please tick yes and rate how stressful the event was.

	Did the event occur?		How stressful was the event for you?		
	Yes	No	Not at all stressful	Moderately	Very stressful
a. Had a lot of tests?					
b. Fought with girlfriend/boyfriend?					
c. Had hassles with teacher / lecturer?					
d. Victim of crime (for example property)					
e. Had housemate conflicts?					
f. Lots of deadlines to meet?					
g. Argument, conflict of values with friend?					
h. Stayed up late writing a paper?					
i. Thoughts about unfinished work?					
j. Problem with transport (e.g. bus, tube)					

**Q4.** The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is don't try to add up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:

- 0 = never
- 1 = almost never
- 2 = sometimes
- 3 = fairly often
- 4 = very often

**In the last month, how often have you.....**

<b>a.</b> been upset because of something that happened unexpectedly?	0	1	2	3	4
<b>b.</b> felt that you were unable to control the important things in your life	0	1	2	3	4
<b>c.</b> felt nervous and stressed?	0	1	2	3	4
<b>d.</b> felt confident about your ability to handle your personal problems?	0	1	2	3	4
<b>e.</b> felt that things were going your way?	0	1	2	3	4
<b>f.</b> found that you could not cope with all the things you had to do?	0	1	2	3	4
<b>g.</b> been able to control irritations in your life?	0	1	2	3	4
<b>h.</b> felt that you were on top of things?	0	1	2	3	4
<b>i.</b> found yourself thinking about things that you have to accomplish?	0	1	2	3	4
<b>j.</b> felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

**Thank you for your help.**





























































